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THE BONE-MARROW VOLUME OF THE ALBINO RAT

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In view of the recent work of Fairman and Whipple ('33) on the bone-marrow volume in adult dogs, which yielded the striking result that the marrow occupies as much space as about two-thirds of the liver, it has seemed worthwhile to make further observations as opportunity offers on other species commonly used in experimental work.

On account of the very tedious labor involved in making measurements of the bone-marrow volume, observations are necessarily limited in number, and it is important, therefore, to choose individual animals which will reasonably represent the average adult of the species. In the case of rats, conventional usage has more or less established the female of 200 gm. as a sort of standard for biological measurement. From a list of the sample weighings of the Wistar experimental colony strain on March 10, 1933, furnished by The Wistar Institute of Anatomy, it appears that the female rat of the Wistar strain attains the weight of 200 gm. at about 115 days. Male rats of the same age average 275 gm. in weight.

By the courtesy of Dr. M. J. Greenman, director of The Wistar Institute, and Miss F. Louise Duhring, curator of the animal colonies, we were furnished with two females, 94 days old at the time of shipment from Philadelphia, and weighing, respectively, 202 and 203 gm., and two males of the same litter weighing, respectively, 279 and 280 gm. These animals were killed when 100 days old, at which time the weights were, respectively, females 180.1, 183.4, and males 253.5, 260.0, the losses in weight having been due undoubtedly to shipment

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and the change of environment. From these data the four animals may be considered fair samples of the average young adult rat.

The determination of marrow volume of these four rats was carried out by a method similar to that described by Töppich ('14) and Nye ('31), and used by Fairman and Whipple on the dog in 1933. The rats were weighed and the skeletons dissected free from soft tissues. Each skeleton was put in a separate jar covered with water and placed in an incubator at 37°C. for 6 days. The water was changed twice a day during this period and a drop or two of ammonia added when necessary to keep the solution neutral. At the end of this time the bones were dropped into Douglas broth and a 5 cc. culture of Clostridium histolyticum (American Type Culture Collection, no. 611) added and incubated in a closed vessel at 37°C. for 6 days. They were autoclaved, then washed in running water during the days and covered with water and incubated at 37°C. each night for 5 days and nights. They were finally washed in running water continuously for 3 days, were dried to constant weight at 47°C., and boiled in fresh ether under reduced pressure for 15 minutes; then allowed to remain in the same ether for 24 hours. This was repeated six times, using freshly distilled ether each time. Each skeleton was dried at 100°C., weighed to constant weight and the weights noted.

It was felt that since the bones were so very small and fragile it would be impossible to wipe off excess agar with gauze. For this reason a thinner agar was used than that used for dogs, and alcohol and ether were used to aid in removal of the excess.

The teeth were discarded. Each skeleton was placed in a small wire gauze basket and dropped carefully into a boiling 2 per cent solution of agar agar. Boiling was continued under reduced pressure for 15 minutes. The agar agar was reheated and the process repeated to insure complete filling of the marrow spaces. The basket of bones was lifted from the agar and dipped quickly into alcohol, ether, hot water,

then iced water. When some of the long bones and the vertebrae were broken and examined carefully with a lens the marrow spaces were found to be completely filled with agar. The water displaced by the bones filled with agar was measured in a 50 cc. graduated cylinder. The cylinder was filled to the 20 cc. mark, the bones of one skeleton dropped in and the rise in water level noted.

To determine the specific gravity of rat bone several of these bones were boiled in water for a half hour. The water was changed and the boiling repeated four times. They were ground in a mortar and sieved to remove the fine bone powder. They were dried to constant weight at 90°C. A known sample of the small fragments was taken in a pyknometer, covered with freshly distilled water and boiled under reduced pressure for 20 minutes to remove all air bubbles. The pyknometer was then filled up with water, carefully dried on the outside and weighed at 22°C. The weight of the bone sample was subtracted from this weight. This result, subtracted from the weight of the pyknometer full of water, gave the volume of the bone sample. Several determinations were made.

The volume of the bones was obtained by dividing the weight of the dry bones by the specific gravity—2.5885. This volume subtracted from the amount of water displaced by the bones filled with agar, gave the volume of the bone-marrow.

The results are tabulated as follows:

TABLE 1

	RAT 1		RAT 2		RAT 3		RAT 4	
	Total weight	Per cent of body weight	Total weight	Per cent of body weight	Total weight	Per cent of body weight	Total weight	Per cent of body weight
Body	183.4		180.1		253.5		260.0	
Marrow	5.86	3.20	5.76	3.23	7.41	2.93	7.04	2.71
Bone	6.05	3.30	6.20	3.41	6.95	2.72	6.85	2.62
Bone + marrow	11.91	6.50	11.96	6.65	14.36	5.59	13.89	5.34

For comparison, table 2 shows the average weights of spleen and blood of rats of comparable size, taken from the graphs given by Donaldson ('24) which are based on measurements of similar rats in The Wistar Institute colony.

TABLE 2

	RAT 1		RAT 2		RAT 3		RAT 4	
	Total weight	Per cent of body weight	Total weight	Per cent of body weight	Total weight	Per cent of body weight	Total weight	Per cent of body weight
Liver	9.3	5.06	9.2	5.11	11.75	4.65	12.1	4.65
Spleen	0.50	0.273	0.48	0.263	0.66	0.260	0.68	0.261
Blood	12.2	6.66	12.1	6.72	14.9	5.90	15.1	5.80

CONCLUSIONS

The method used is obviously such as to give a less accurate result with animals such as the rat, having very small bones, than with the dog, but the approximate agreement of the measurements of bone-marrow volume obtained from the four rats and the fact that they are of the same order of magnitude as in the dog suggest that they are at least approximately correct and of definite usefulness for studies in erythropoiesis in the rat.

As in the dog, the bone-marrow volume is surprisingly large, about 3 per cent of the body weight, and, therefore, about three-fifths of the liver weight.

LITERATURE CITED

DONALDSON, H. H. 1924 The rat. Data and reference tables for the albino rat. 2nd ed., Philadelphia.

FAIRMAN, EDNA, AND G. H. WHIPPLE 1933 Bone marrow volume in adult dogs. Am. J. Physiol., vol. 104, pp. 352–357.

NYE, R. N. 1931 Bone marrow volume in rabbits. Proc. Soc. Exp. Biol. and Med., vol. 29, pp. 34–37.

TÖPPICH, G. 1914 Die Porosität der Knochen des Neugeborenen mit Berücksichtigung des Verhaltens der Porosität bei Erwachsenen und Greisen. Arch. f. Anat. u. Physiol., anat. Abt., Bd. 38, S. 9–24.

THE EFFECT OF ELEVATED TEMPERATURE ON THE MITOCHONDRIA OF THE GUINEA PIG PANCREAS

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Although the recent investigations of Bensley and Gersh on the chemical nature of mitochondria have placed the matter in a new light, interest still attaches to the several reports which indicate a rather sharply defined effect of exposure to elevated temperatures upon the visibility of the granules, and for this reason the results of some carefully controlled experiments on one particular tissue are here reported.

The mitochondria in the fresh kidney and liver of frogs were found by A. Policard in 1912, to disappear after exposure to temperatures of 47° to 50°C. for 10 to 30 minutes followed by fixation in Regaud's bichromate-formol solution and staining by iron-hematoxylin. In contrast, M. R. Lewis and W. H. Lewis found (by direct observation on the warm stage) that in chick embryo tissue cultures the mitochondria did not disappear even after heating at such temperatures for 1 hour. In 1917 N. H. Cowdry, working with the guinea pig pancreas and the radicle of the pea along lines laid down by Policard, found that in both tissues the mitochondria disappeared at 48° to 50°C. on exposure for 20 to 30 minutes. Finally Policard and Mangenot ('22), working with filaments of the alga Saprolegnia and the epidermal cells of iris and tulip leaves, again found that the mitochondria disappeared suddenly upon exposure to temperatures from 48° to 50° for 10 minutes. This was done both by direct observation of the fresh tissue upon the warm stage and by study of the fixed tissue after exposure to the various temperatures.

In order to confirm these various authors, it was decided to repeat the work on the guinea pig pancreas, both by direct observation of the fresh tissue and by fixation and staining of previously fixed tissue. The experiments were performed as follows:

A young, recently fed guinea pig was killed by a blow on the head; the pancreas was at once carefully removed and small pieces, not more than 3 mm. thick, were cut from it. These pieces were placed into tubes at the bottom of which were pieces of cotton moistened with saline. Each tube was provided with a thermometer and was immersed in a heated water bath. The length of exposures are given in the table. At the end of each period, the bits of tissue were placed in Regaud's bichromate-formol solution. Staining was by iron-hematoxylin. For the direct observation of the mitochondria in acinar cells, small bits of pancreas from the freshly-killed animal were teased in saline on a slide and covered by a cover glass ringed with vaseline. Here the heat was supplied by a warm box, the thermometer being 2 mm. from the surface of the cover glass.

By direct observation the mitochondria began to be indistinct at 20 minutes and disappeared completely at 30 minutes from 47° to 48°C. This corresponded exactly with the fixed and stained sections, the results of which are given in the table.

	Control	*40°C.*	*45°C.*	*47°C.*	*50°C.*	*55°C.*
10 minutes	+	+	+	+	0	0
20 minutes	+	+	+	+	0	0
30 minutes	+	+	+	0	0	0

+ Indicates presence of mitochondria.

Other pieces of tissue were first subjected to the usual Regaud fixation and then exposed to high temperatures. The exposure of such tissue to 50°C., 100°C., 150°C., and 200°C. for a period of ½ hour produced no evident change in the mitochondria.

CONCLUSIONS

1. By direct observation of surviving tissue and by fixation and staining of previously fixed tissue, the mitochondria of the guinea pig pancreas are found to disappear in 20 to 30 minutes at 47°C. This confirms previous work.

2. In tissue previously fixed in potassium bichromate-formol solution, temperatures of 200°C. have no effect.

LITERATURE CITED

BENSLEY, R. R., AND I. GERSH 1933 Studies on cell structure by the freezing-drying method. Anat. Rec., vol. 57, pp. 205–233.

COWDRY, N. H. 1917 A comparison of mitochondria in plant and animal cells. Biol. Bull., vol. 33, pp. 196–228.

LEWIS, M. R., AND W. H. LEWIS 1915 Mitochondria (and other cytoplasmic structures) in tissue cultures. Am. J. Anat., vol. 17, pp. 339–401.

POLICARD, A. 1912 Sensibilité des chondriosomes aux élévations de température. C. R. Soc. Biol., T. 72, pp. 228–229.

POLICARD, A., AND G. MANGENOT 1922 Action de la température sur le chondriome cellulaire. Un critérium physique des formations mitochondriales. A. R. Acad. des Sciences, T. 174, pp. 645–647.

UTERINE BLEEDING IN A BAT, GLOSSOPHAGA SORICINA

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TWO PLATES (FOURTEEN FIGURES)

Uterine bleeding of one sort or another has long been known to occur as a normal feature in the reproductive cycle of many animals. Within recent years it has been demonstrated that there are two types involved, a bleeding associated with oestrus which is found in many species, and the menstrual bleeding of the higher primates. Lately a third type has been described, the so-called 'placental sign' of rat and monkey, where a small amount of blood is discharged during the early phases of placentation. Up to the present time no actual description has been given of any of these phenomena in the bats, although Kohlbrugge states that one of the flying-foxes (Xantharpyia) 'menstruates.' I have accordingly thought it worthwhile presenting the following description and photographs of a well-marked uterine bleeding found in Glossophaga soricina of the South American family Phyllostomidae.

The material described in this paper was collected in eastern Matto Grosso, Brazil, in November and December, 1933. The embryology is being described in another paper; certain features must be mentioned here, however, as having a possible bearing on the bleeding. The uterus is simplex with no trace of cornua. Implantation is at the fundus, and differs from all other bats in that the ovum burrows completely into the endometrium after the fashion of the human ovum, so that a typical set of decidual membranes is formed. In prepa-

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ration for this burrowing implantation there occurs a marked post-ovulatory thickening of the endometrium, but I am not prepared to say whether or not this is excessive as compared to the other bats in which implantation is superficial. Twelve non-pregnant uteri from adult animals are available, the data being summarized in the accompanying table.

TABLE 1

SERIAL NUMBER	OVARIES	UTERUS
1 B 59	No corpus luteum	A few leucocytes in lumen
1 B 61	No corpus luteum	Cavity empty
1 B 71	No corpus luteum	Leucocytes
1 B 75	No corpus luteum	A few leucocytes in cervix
1 B 81	No corpus luteum	Cavity empty
1 B 56	No corpus luteum	Leucocytes, erythrocytes and cellular debris
1 B 74	Corpus luteum present	Endometrium very thick, glands hypertrophied, no bleeding
1 B 53	Corpus luteum present	Leucocytes, erythrocytes and cellular debris
1 B 68	Corpus luteum present	Leucocytes, erythrocytes and cellular debris
1 B 72	Corpus luteum present	Leucocytes, erythrocytes and cellular debris
1 B 57	Corpus luteum present	Leucocytes, erythrocytes and cellular debris
1 B 55	Corpus almost disappeared	Leucocytes, etc., reduced in number, endometrium returning to normal

The first five uteri listed in the table give us a picture of the non-pregnant organ prior to ovulation. Three of these are reproduced in figures 1 to 3. All are frontal sections and are oriented with fundus above, cervix below. The thin endometrium and poorly developed glands are characteristic of this stage. Uterus 81, shown in figure 3, is unique in having its lumen greatly dilated. This may be a pro-oestral change. In figure 2 two masses of leucocytes may be seen at the fundal end of the lumen. I am unable to give an explanation for their presence in the lumen in such numbers; a similar migration of leucoyctes into the cavity may be found at any stage, both in non-pregnant and in pregnant animals.

The next stage in the uterine cycle is represented by uterus 74 (fig. 5). There is a corpus luteum in one ovary but no egg can be found either in tube or in uterus. The endometrium is

much thicker than in the previous stage and the glands are longer and more or less crooked. The greatest development is in the fundal region, where implantation ordinarily takes place. This hypertrophied endometrium corresponds to the pre-gravid or pre-menstrual endometrium described for many other species.

The last five animals (figs. 6 to 10) show the uterine bleeding mentioned at the beginning. Each of the five has a corpus luteum in one ovary, but none shows an ovum or embryo. In the first four of these the uterus is seen to be slightly larger than the 'pre-menstrual' stage of figure 5, and much larger than the uteri before ovulation. The last one, figure 10, is a later stage, as shown by the corpus, and the uterus is returning to the di-oestral size and condition. In all five the hypertrophied endometrium of the fundus is seen to be disintegrating to a greater or less degree, and the lumen is partly filled by a mass which is somewhat continuous with the sloughing endometrium. An examination of this mass under higher power (figs. 11 to 14) shows it to be made up of three elements. First and most conspicuous is a ground substance which seems to be partly hemolyzed and coagulated blood, the individual corpuscles being recognizable here and there where they have not yet broken down. Embedded in this coagulum are vast numbers of leucocytes together with single cells, cell masses, and whole fragments from the endometrium itself. The figures give a better idea of the appearance of the composition than any description.

Let us now review briefly the successive changes in the uterus. Before ovulation the endometrium is thin (figs. 1 to 3). After ovulation and the formation of a corpus luteum, the endometrium thickens greatly in preparation for implantation (fig. 5). But if the egg is not fertilized, or if it dies, the hypertrophied endometrium disintegrates and sloughs off, with a marked extravasation of leucocytes and blood (figs. 6 to 10). This last phenomenon, when found in the higher primates, is called menstruation. Whether it should be called that here is perhaps open to question. As found in ape or in

human, menstruation is part of a monthly cycle. In Glossophaga there is apparently but one oestrous cycle, and hence never more than a single bleeding, per year. If the physiologists wish to restrict the term to the monthly cycle of the primates, I shall not quarrel with them. Only, it must be emphasized that the bleeding found in the bat is not only homologous to menstruation, as hitherto defined, but is actually identical with it except in point of frequency.

Of the twelve non-pregnant uteri examined, eleven fit perfectly into the series of events described above. The twelfth is somewhat anomalous, and I am not certain as to its interpretation. This is no. 56, shown in figure 4. The ovaries are exceptionally large and are crowded with follicles whose zona granulosa is very thick. No corpus, nor any trace of a recently ruptured follicle, can be found. The uterus, as may be seen from the figures, is smaller than the typical menstruating organ and the endometrium is not so thick; nevertheless this specimen appears to be menstruating. The 'clot' is similar in composition to those of figures 11 to 14.

Quite recently it has been demonstrated, notably by Hartman, that in the macaque menstruation may take place without a preceding ovulation. I venture to suggest that possibly the bleeding in bat 56 may be homologous to the summer menstruations (without ovulation) of the monkey. The ovaries of this individual are certainly not like those of a typical di-oestral or pro-oestral animal, and doubtless more abundant material would allow us to explain this case on a well-established hormonal basis.

As was mentioned previously, the only reference I have found to 'menstruation' in the bats is in two papers by Kohlbrugge on Xantharpyia. That this author was dealing with true menstruation is, I think, questionable. No figures are shown of the process, but in one place he speaks of the 'menstruating' uteri as containing lymphocytes and mucus, and makes no reference to the presence of actual blood, nor to any destruction of the endometrium. Certainly this would not seem to be the same as the bleeding and 'sloughing in-

volved in true menstruation. Furthermore, he states expressly that the phenomenon bears no relation to conditions in the ovaries, and that one horn (of the duplex uterus) may be in 'menstruation' while the other contains an embryo. I believe that Kohlbrugge was dealing with an effusion of leucocytes, such as is shown in figure 2, and not with a true menstrual change. Further study, with this question in mind, of some species of this family will be necessary to settle this point.

LITERATURE CITED

HARTMAN, CARL G. 1932 Studies in the reproduction of the monkey Macacus (Pithecus) rhesus, with special reference to menstruation and pregnancy. Carnegie Contrib. Embryol., vol. 23, pp. 1–161.

KOHLBRUGGE, J. H. F. 1904 Das bei der Menstruation ausgestossene Ei. Zeitsch. f. Morph. u. Anthrop., Bd. 12, S. 579–585.

———— 1913 Befruchtung und Keimbildung bei der Fledermaus 'Xantharpya amplexicaudata.' Verh. d. K. Akad. van Wetensch. te Amsterdam, Sect. 2, Deel 17, S. 1–37.

PLATE 1

EXPLANATION OF FIGURES

Frontal sections through uteri. All figures × 22.

1 to 3 Uteri 1 B 61, 71, and 81, respectively. No corpus luteum in these three animals. Note the thinness of the endometrium in all three. In figure 2 there are two groups of leucocytes at the fundus, otherwise the uterine cavities are practically empty.

4 Uterus 1 B 56. There is no corpus luteum, but the endometrium is slightly thickened and the cavity is filled by blood and sloughed epithelium.

5 Uterus 1 B 74. Corpus luteum in ovary. Endometrium very thick. No sign of menstruation as yet.

6 to 10 Uteri 1 B 53, 68, 72, 57, and 55, respectively. Corpus luteum present in each animal. These are menstruating, and in each case the uterine cavity is partly filled with blood and cellular debris. In figure 10 the process seems to be near its end, and the corpus luteum in this animal has almost disappeared.

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PLATE 2

EXPLANATION OF FIGURES

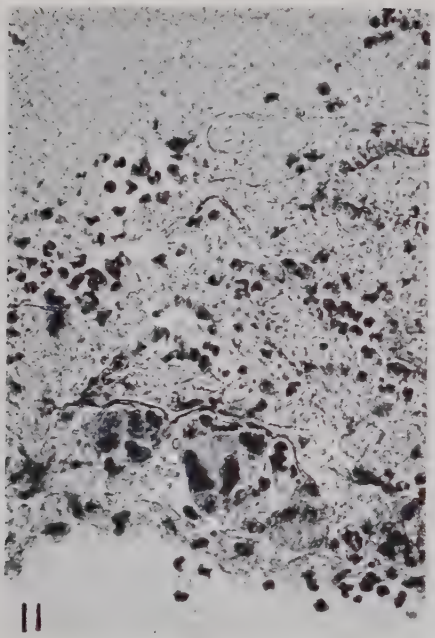
Parts of the menstrua shown in the uteri in plate 1. All figures × 450.

11 Enlargement of figure 6. Menstrum principally blood, leucocytes in center of field, several fragments of uterine epithelium below.

12 Portion of figure 7 enlarged. Above, partly dissociated endometrium; below, a sloughed fragment. Numerous leucocytes throughout field.

13 Enlarged from figure 9. Menstrum largely blood, with darkly staining epithelial cells in masses and scattered uterine cells of another type (connective tissue ?). Mass of leucocytes at lower right.

14 Taken from figure 10. Above, sections of uterine glands with infiltration of leucocytes around them. Below these, blood and leucocytes; detached masses of endometrium at bottom.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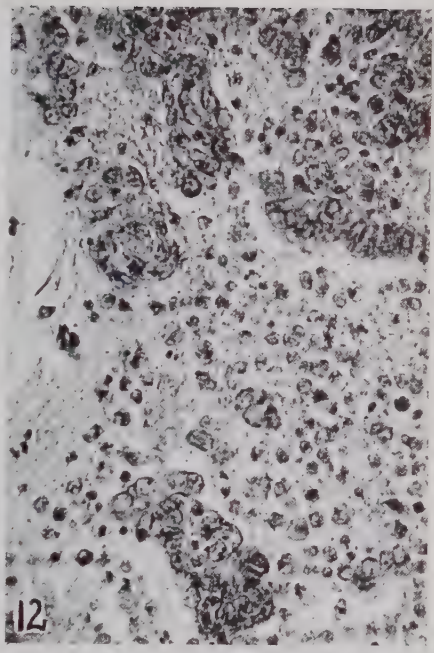
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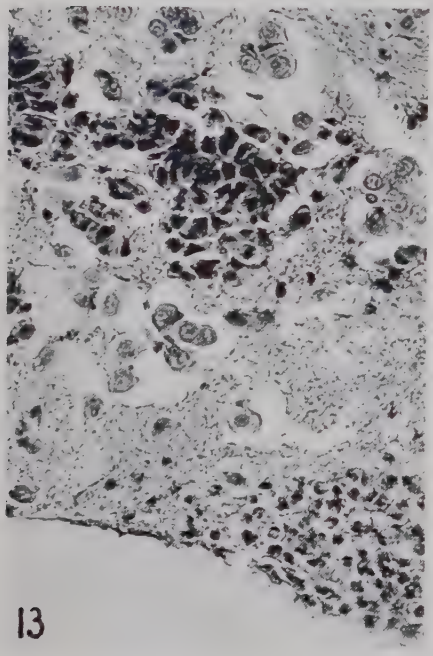
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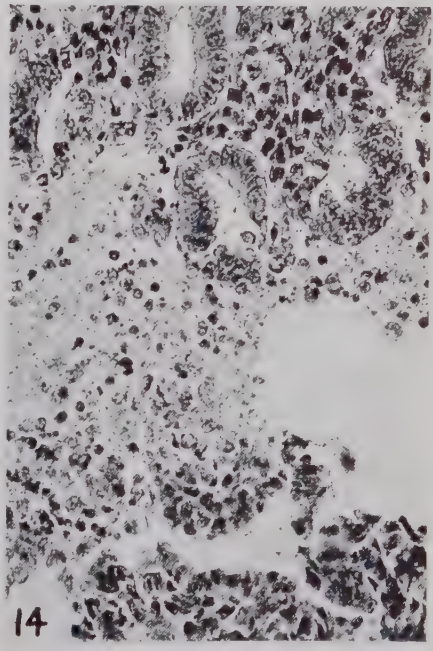
14

AN INEXPENSIVE COARSE ADJUSTMENT REMOTE CONTROL FOR PHOTOMICROGRAPHY

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ONE PLATE (THREE FIGURES)

In the horizontal type of photomicrographic camera, focusing is difficult owing to the distance between the microscope and the focusing screen of the camera. On most microscopes, this difficulty in the case of the fine adjustment is easily overcome by passing a thread over the adjustment wheel (figs. 2 and 3). This method is not suitable for the coarse adjustment, however, and the apparatus described here is to overcome this difficulty. It can be made quite inexpensively and can be removed from the microscope in a few moments by taking out four screws.

The apparatus was of course specially made to fit the microscope used for photomicrography here, but the ideas embodied in it could be used on any microscope. The bevel gears employed are sold commercially as Meccano gears and are quite satisfactory, though a smoother action could be attained with more accurately cut wheels. The pair used gave a reduction of three to one. The original hub in the large bevel has been replaced with one (figs. 1 and 2, E) in which the hole is tapered to fit the spindle of the coarse adjustment. One of the radial holes in the wheel has been enlarged to allow access to a screw holding the apparatus to the body of the microscope.

The body (fig. 1, A) of the apparatus can be made of a brass casting $\frac{1}{8}$-inch thick, or with two pieces of brass silver-soldered together. The first piece bolts to the microscope and the second piece, soldered at right angles to it, holds the bearing for the small bevel (fig. 1, B).

The remote control shaft (fig. 1, F) is a steel rod $\frac{1}{8}$-inch in diameter of any length required. At one end there is a knob with which it can be turned and at the other end it is connected by a brass sleeve to about 6 inches of flexible speedometer cable (fig. 1, C). The other end of the cable is soldered into another sleeve which pushes on to the shaft of the small bevel (figs. 1 and 2, D).

This apparatus has been used with great success with magnifications up to 200. Above this, critical focus was obtained by means of the thread on the fine adjustment.

PLATE 1

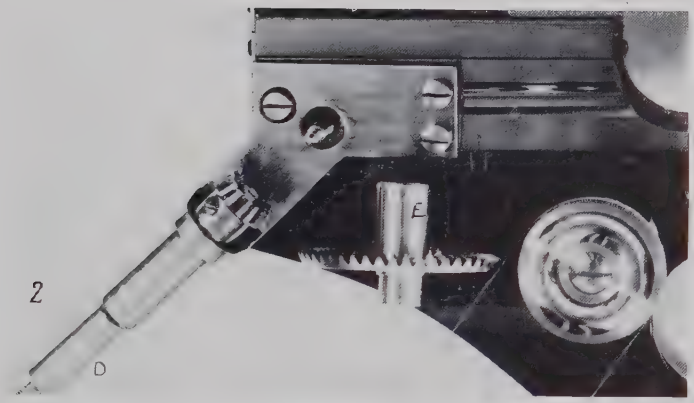
EXPLANATION OF FIGURES

1 Component parts of apparatus. A, body of apparatus; B, bearing for small bevel; C, flexible speedometer cable; D, sleeve to fit shaft of small bevel; E, hub with tapered hole to fit coarse adjustment spindle; F, remote control shaft.

2 Apparatus on microscope with large bevel removed from spindle.

3 Apparatus completely assembled.

1



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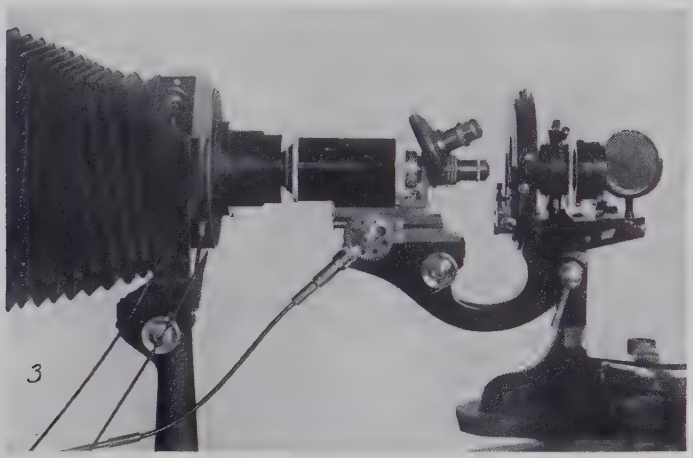

3

PRENATAL GROWTH OF THE CAT

IV. GROWTH IN LENGTH AND WEIGHT OF THE DIGESTIVE TUBE

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SIX FIGURES

This paper will give the growth in weight and in length of the digestive tube and of its divisions with reference to total body weight and to nose-anus, or body, length. The same specimens were used in this study as in the preceding three papers and so the description of the material will not be repeated here. The digestive tubes of three fetuses were accidently injured and were not studied. This leaves a total of 226 fetuses and thirty-five newborn kittens used in this study.

Very little has been found in the literature on the prenatal growth of the digestive tube. Jackson ('09) cites the earlier papers and gives the weights of the stomach and intestines in the human fetus. Scammon and Kittelson ('26) give the linear growth of six parts of the digestive tube of the human fetus. The growth in weight of the stomach and intestines has been studied in the pig fetus by Lowrey ('11), and in the embryonic and young dogfish by Kearney ('14).

The head of each fetus had been removed as described in the first paper of this series. This incision passed through the top of the larynx thus separating the esophagus from the pharynx. In the dissection of the viscera, the entire tube, from upper end of the esophagus to the anus, was removed, freed from the adjacent fat, connective tissue and mesenteries, and weighed in a glass stoppered weighing bottle. In this study the 'digestive tube' includes the esophagus, stomach,

small and large intestines. The glands of the digestive system are not included, but will be presented in a later paper. The entire tube was laid on a dissecting pan and straightened as much as possible and the total length measured with a steel tape graduated to millimeters. Next, the stomach was separated from the esophagus and the small intestine, and then the small and large intestines were separated. All of these incisions were made at well-defined landmarks. The constriction of the pylorus marked the place for separation of stomach and small intestine. Each of these subdivisions of the tube was weighed in a glass stoppered weighing bottle and the lengths of the esophagus and the two intestines were measured in a manner similar to that used for the entire tube. The tube and its divisions were kept in a moist chamber all of the time except when being measured or transferred to the weighing bottles. All of the weights were made on a chemical balance sensitive to one-tenth of a milligram.

The contents of no part of the tract were removed. In the smaller specimens there was little in the tube and it would have been very difficult to have removed it without seriously injuring the tube. In the older fetuses and newborn specimens there was an increasing amount of meconium. Some of the newborn kittens had sucked and their stomachs contained milk which had been coagulated by the formalin preservation. In the older fetuses and newborn cats there was more material especially in the large intestine. This will be discussed later in connection with these subdivisions of the tube.

To save space no tables will be given. The formulae for all curves afford a means of determining the average weight or length of the tract or any subdivision, for any body length or body weight, within the specified limits of the formulae. All of the formulae were derived in the same manner as in the previous numbers of this series (see no. III).

GROWTH IN WEIGHT

The growth in weight of the entire tube and of its four divisions is shown in figure 1. The individual cases are shown

only with the curve of growth for the entire tube. Condensing all five curves in one figure prevents the showing of the individual cases for each curve but it has the advantage of making possible the comparison of the curves of the parts since all are drawn to the same scale and in the same figure. The curve rising rapidly at first and then more slowly and marked '%'

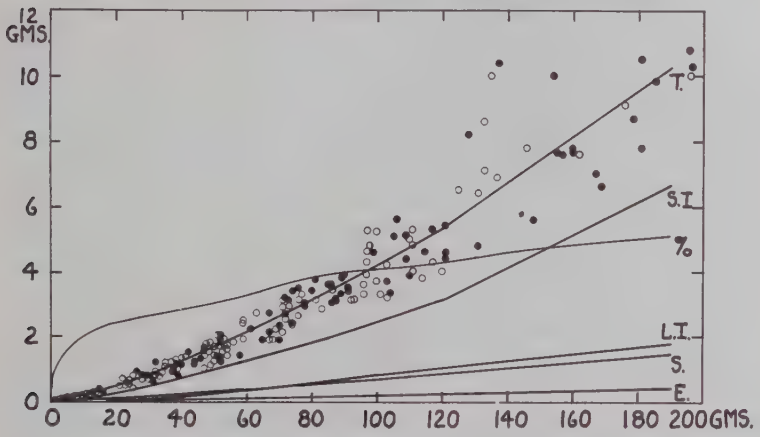

Fig. 1 Growth in weight of the digestive tube and of its parts plotted on body weight. The upper curve (T.), with the males represented as dots and the females as circles, gives the weights of the entire tube. Below are the small intestine (S.I.), large intestine (L.I.), stomach (S.) and esophagus (E.). Formulae from which these curves were drawn are given in the text. The curve marked (%) gives the weights of the entire tube reduced to percentages of the body weight. The values in the left margin give both the weights and percentages.

gives the weights of the entire tube expressed as percentages of the total body weight.

The empirical formulae from which all of these curves of absolute growth were drawn are as follows:

Entire tube (T.), from 0.6 to 120 gm. of body weight,

$Y = 0.01\ (X^{1.31})$

and from 120 to 190 gm. of body weight,

$Y = 0.07\ (X-120) + 5.293$

Small intestine (S.I.), from 0.6 to 120 gm. of body weight,

$Y = 0.01\ (X^{1.25}) - 0.007X$

and from 120 to 190 gm. of body weight,

$Y = 0.05\ (X-120) + 3.13$

Large intestine (L.I.), from 3 to 95 gm. of body weight,
$Y = 0.000035\ (X^{2.2}) + 0.0054$
and from 95 to 190 gm. of body weight,
$Y = 0.0105\ (X–95) + 0.800$
Stomach (S.), from 4 to 80 gm. of body weight,
$Y = 0.01\ (X^{1.13}) — 0.0113X + 0.016$
and from 80 to 190 gm. of body weight,
$Y = 0.0085\ (X — 80) + 0.5261$
Esophagus (E.), from 1 to 120 gm. of body weight,
$Y = 0.001\ (X^{1.14}) — 0.00005X + 0.002$
and from 120 to 190 gm. of body weight,
$Y = 0.00256\ (X — 120) + 0.2305$

In all of these formulae Y represents the weight in grams of the tube or its subdivisions and X represents the gross body weight also in grams. There is no apparent sex difference in the distribution of the cases for the tube or any of its parts. All of the cases of the weight of the entire tube above 121 gm. of body weight are newborn cats. The greater scatter of these cases may be explained by the variable amount of material in the stomach and intestines.

The average weight or length of each part was found and likewise the average weight or length of the body, for each ten units of the abscissae. Using the empirical formulae, the weight or length of each part was computed for each ten-unit value of the body weight, or body length, and the average of the differences between these observed and calculated values, without respect to sign, was obtained for each curve. This gives some idea of the goodness of fit of the curves and makes it possible to compare the curve with the cases shown and the other curves. The average difference between the observed and the calculated values of the entire tube is 8.43 per cent. The average of all of these averages for figure 1 is 7.78 per cent. The esophagus has the lowest average difference, or 4.73 per cent, and the highest average difference in this group, and for all of the curves presented in this paper as well, is 10.50 per cent for the large intestine.

The esophagus is the lightest part of the tube throughout most of the period. The average weights of these divisions of the tube in the specimens with body weights less than 1 gm. show that the large intestine is the lightest, averaging but

0.0008 gm. This is followed by the esophagus (0.0010 gm.), small intestine (0.0013 gm.) and the stomach (0.0035 gm.) is the heaviest subdivision. At 4 gm. of body weight the order is unchanged except that the small intestine with a weight of 0.0286 gm. has surpassed the stomach (0.0187 gm.) and thereafter it is the heaviest part of the digestive tube. By the time the specimens have reached a body weight of 30 gm. the large intestine has passed the esophagus in weight and thereafter the esophagus is the lightest part of the tube. The large intestine is lighter than the stomach until a body weight of 80 gm. is attained and then the stomach is next to the lightest subdivision of the tube. These relations do not show well in figure 1 due to the small scale used in drawing the lighter parts of the tube. Thus we find the large intestine, which was lightest at first, forming next to the largest part at birth; the esophagus started next to the lightest and it is the lightest in the newborn. The stomach was heaviest at first and in the newborn it is next to the lightest while the small intestine, which was next to the heaviest at first, is by far the heaviest at birth.

Using the observed weight, or the average weight of the group under 1 gm. of body weight, since some of the formulae do not fit this smallest group, and determining the total increase up to 190 gm. of body weight, we find the following results. The entire digestive tube increases 1544.4 times in weight during this growth period while the body weight increases but 302.5 times or but about one-fifth as rapidly. The esophagus and stomach increase less than the entire tube, or 410 and 418 times, respectively. Both of these increases are greater than the total increase in the body weight. The two intestines increase more than the entire tube, or 2248 times for the large intestine and 5100 times for the small intestine. Thus the greatest increase in prenatal growth is in the small intestine and the least in the esophagus.

The weight of each digestive tube was reduced to a percentage of the gross body weight. These percentages were plotted on a preliminary chart and a curve drawn by inspec-

tion through the cases and this curve without the cases transferred to figure 1 is shown as the percentage curve. This shows the digestive tube increasing from about 1 per cent to a little over 5 per cent of the gross body weight during this prenatal growth period. This is in accord with the findings in the preceding paragraph in which it was shown that the total increase in the weight of the digestive tube was little more than five times as great as that for the gross body weight. Jackson ('09) finds that the stomach and intestines (with contents) of the human fetus form 1.28 per cent of the body weight in the second fetal month and this increases to 4 per cent in the newborn. Lowrey ('11) gives a curve for the percentage weight of the stomach and intestines of the pig fetus, which is much like the one shown here except that his values rise to a somewhat higher level (6 per cent) in the newborn.

To better show the changes in the proportions of the weights of the divisions of the tube, the percentage weight of each part relative to the weight of the entire tube was determined. These percentages were plotted on a preliminary chart, curves drawn through the averages for each 10 gm. of body weight increase and the resulting values were used in constructing figure 2. The areas in figure 2 represent the relative weights of the divisions of the entire digestive tube, throughout the fetal growth period. The individual percentages were grouped very closely about the curves on the preliminary charts. These preliminary curves show even better than in figure 2, the very early and precipitous drop in the percentages of the esophagus and stomach and a similar marked rise in the percentages of the small intestine. The initial changes in the large intestine were much less marked. The esophagus drops from an initial percentage of 15 to a little over 4 per cent in the largest specimens. The stomach decreases from 52.5 per cent to 15 per cent. The small intestine increases from 20.5 to 63 per cent and the large intestine from 2 per cent to 18 per cent.

These same percentage weights of the divisions of the digestive tube were plotted on body length as abscissae and a diagram was prepared in the same manner as figure 2. In this chart, not shown, the initial changes were prolonged over a longer period. The grouping of the individual percentages on the preliminary charts was not as close about the curves plotted on body lengths as it was when plotted on body weights as abscissae.

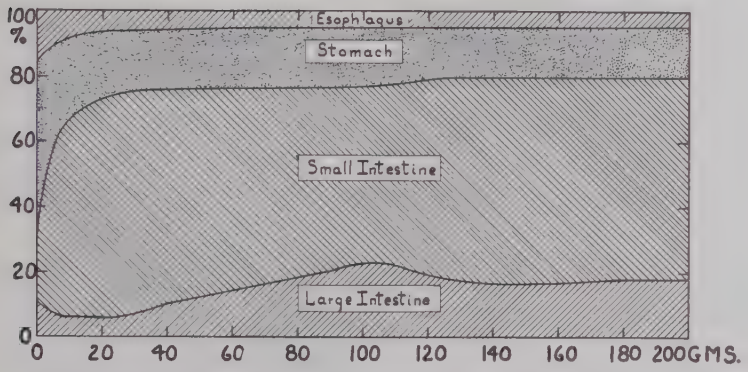

Fig. 2 Each area represents the percentage weight of a part of the tube expressed in percentages of the weight of the entire tube and plotted on body weight.

The growth in weight of the tube and its four subdivisions, plotted on body length is shown in figure 3. The empirical formulae for these are as follows:

Entire tube (T.), from 30 to 140 mm. of body length,

$$Y = 0.000026\ (0.1X)^{4.2}$$

and from 140 to 190 mm. of body length,

$$Y = 0.068\ (X - 140) + 1.693$$

Small intestine (S.I.), from 40 to 140 mm. of body length,

$$Y = 0.0000076\ (0.1X)^{4.5} - 0.0033$$

and from 140 to 190 mm. of body length,

$$Y = 0.038\ (X - 140) + 1.089$$

Large intestine (L.I.), from 30 to 140 mm. of body length,

$$Y = 0.00000136\ (0.1X)^{4.5} + 0.00016$$

and from 140 to 190 mm. of body length,

$$Y = 0.024\ (X - 140) + 0.1956$$

Stomach (S.), from 30 to 140 mm. of body length,

$$Y = 0.0000227\ (0.1X)^{3.6} + 0.0006$$

and from 140 to 190 mm. of body length,

$$Y = 0.013\ (X - 140) + 0.304$$

Esophagus (E.), from 30 to 140 mm. of body length,

$$Y = 0.000012\ (0.1X)^{3.4}$$

and from 140 to 180 mm. of body length,

$$Y = 0.01\ (0.01X)^{5} + 0.001X - 0.0992$$

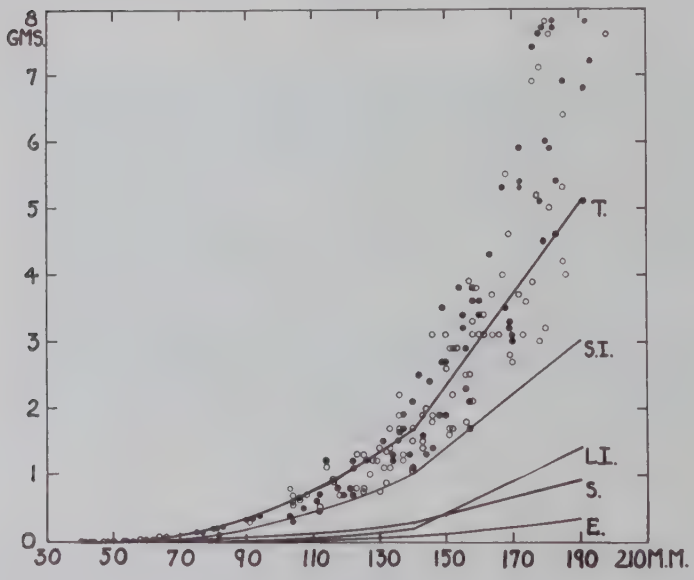

Fig. 3 Growth in weight of the tube and of its parts plotted on body length. The upper curve (T.). with the males represented as dots and the females as circles, gives the growth of the entire tube. The lower four curves are: small intestine (S.I.), large intestine (L.I.), stomach (S.), and esophagus (E.). The formulae are given in the text.

In all of the above formulae Y represents the weight in grams of the digestive tube or of its part and X represents the body length, or nose-anus length, measured in millimeters. The cases are given for the weights of the entire tube and only the curves for the divisions are shown as was done in figure 1.

The individual weights of the digestive tube for the newborn specimens do not group themselves about the curve in figure 3 but are definitely above it. All of the cases above

5.3 gm. in weight are for the digestive tube of newborn kittens. This condition is found for all of the subdivisions of the tube except the esophagus. It is very likely due to the increase in the contents of the stomach and the small and large intestines mentioned above. In fitting these curves no attempt has been made to fit the cases of these newborn kittens except for the weights of the esophagus. The average percentage difference between the observed and the calculated values, as described above, is 8.72 per cent for the weight of the entire tube. This is the largest percentage difference found in this group of curves. The smallest average difference is 5.55 per cent for the stomach curve. The average for the five curves shown in this figure is 7.54 per cent, or just a little less than for these weights plotted on body weight (fig. 1). The weights of the tube and its parts of the newborn kitten are not included in these averages.

Using the body lengths as abscissae, as has been shown in the earlier papers of this series tends to scatter the smaller specimens so that they may be studied better but it crowds the larger specimens, and in the weights of the digestive tract it segregates the fetuses and the newborn specimens, at least in the study of the weights of the entire tube and all of its parts except the esophagus. If the contents of the tube had been removed this might not have happened. This elongation of the earlier portion of the growth by use of body lengths as abscissae probably accounts for the apparent delay in the surpassing in weight of the stomach by the large intestine which does not occur until about 150 mm. of body length. This occurs at about 80 gm. of body weight as shown in figure 1.

All of these curves of absolute growth based on either body weight or body length are concave superiorly for the first part of the curve at least. This shows that the tract and its parts increase in rate of growth during this period.

GROWTH IN LENGTH

The increase in length of the digestive tube and of its divisions plotted on body weight is shown in figure 4. The empirical formulae from which these curves of absolute growth were drawn are as follows:

Entire tube (T.), from 1 to 190 gm. of body weight,
$Y = 47 (X^{0.55}) - 0.66X + 5$
Small intestine (S.I.), from 1 to 100 gm. of body weight,
$Y = 42 (X^{0.5}) - 0.2X - 16.0$
and from 100 to 190 gm. of body weight,
$Y = 1.8 (X - 100) + 384.0$
Large intestine (L.I.), from 0.6 to 140 gm. of body weight,
$Y = 7 (X^{0.5}) - 0.05X$
and from 140 to 190 gm. of body weight,
$Y = 0.123 (X - 140) + 75.824$
Esophagus (E.) from 1 to 60 gm. of body weight,
$Y = 13 (X^{0.3}) - 2.0$
and from 60 to 190 gm. of body weight,
$Y = 0.21 (X - 60) + 42.395$

In all of these formulae Y represents the length in millimeters of the digestive tube or its part and X represents the body weight in grams. The individual cases are shown for only the length of the entire tube and as in the preceding figures only the curves are given for the segments of the tube. The lengths of the stomachs were not measured.

The average of the differences between the calculated and the observed values for the lengths of the digestive tube on body weight is but 2.70 per cent. This is the smallest percentage of any of this group. The average for all four curves is 3.02 per cent, with a maximum percentage difference of 3.33 for the small intestine. These curves in this figure have the lowest average difference of any of the groups. The group having the next lowest average percentage difference is the group giving the lengths of the tube and its parts on body length, shown in figure 6, and the two groups of the weights of the tube and its parts have the two highest average differences. The small intestine has the greatest length at 0.6 gm. of body weight, or 8.62 mm., and it remains the longest division up to birth. In the smallest specimens the large

intestine averages 5.39 mm. and the esophagus 8.37 mm. The same relative lengths of these two divisions are maintained until a body weight of between 10 and 20 gm. is attained and then the esophagus becomes the shortest part of the digestive tube. The changes in the lengths of the small and the large intestines, compared to each other, were studied for

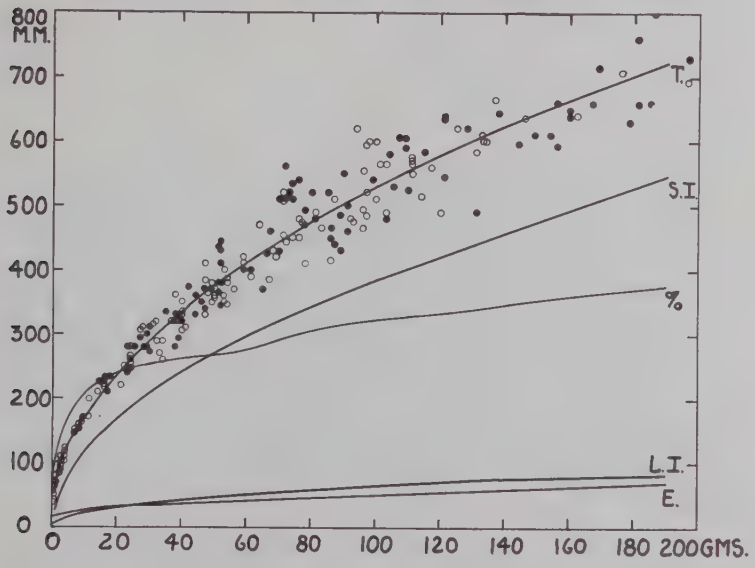

Fig. 4 Growth in length of the tube and three of its divisions. The upper curve (T.), with the males shown as dots and the females as circles, gives the increase in length of the entire tube. Below are the curves for the small intestine (S.I.), large intestine (L.I.) and esophagus (E.). The formulae used in drawing these curves are given in the text. The percentage curve (%) gives the length of the tube expressed as a percentage of the body length with the values in the left margin giving the percentages as well as millimeters.

each 10 gm. increase in body weight, using the computed values. At 0.6 gm. of body weight the small intestine is 1.60 times the length of the large intestine. At 10 gm. of body weight this has increased to 5.27 times and thereafter the increase is slower, but uniform, until at 190 gm. of body weight the small intestine is 6.66 times the length of the large intestine.

The total increase in the length of the tube and of its parts is as follows: digestive tube, 27.4 times; esophagus, 8.34 times; large intestine, 15.21 times and the small intestine increases 63.34 times. During the same growth period the total body length increases but 6.67 times. Thus the esophagus increases less than the total tube increase just as was shown for its increase in weight. The length of the large intestine increases less than the total tube increase but it increases in weight more than the total increase for the entire tube weight as shown above. The small intestine increases in length more than the entire tract just as its weight increases more than any part of the entire tube. The length of the tube and all of its parts increases more than the increase in body length.

The lengths of the entire digestive tube given as percentages of the length of the body are shown in the curve marked '%' in figure 4. The numbers in the left margin give the percentages for this curve as well as the lengths in millimeters. The tube forms but 50 per cent of the body length in the smallest specimens. This means that in the smallest specimens the digestive tube is practically a straight tube extending from the mouth to the anus while the body length, or the nose-anus length, was measured along an arc of a circle from nearly the same two points. This percentage rises rapidly showing that the digestive tube increases in length more rapidly at first than the body length. Its later rate of increase is slower but it finally reaches a length of 375 per cent of the nose-anus length in the newborn kittens.

The relative proportions of the lengths of the parts of the digestive tube are shown in figure 5. The lengths of each division of the tube expressed as percentages of the length of the entire tube were plotted on body weight on a preliminary chart as was done for the percentage weights of the divisions of the tube in the preparation of figure 2. The average values of these percentages were used in preparing figure 5. The stomachs were weighed but the lengths were not measured, hence the lengths of the stomach shown here are really the total length of the tube minus the sum of the

lengths of the esophagus, small and large intestine. If the lengths were determined as accurately as the weights shown in figure 2, this area in figure 5 gives a fairly accurate idea of the stomach length.

The percentage length of the large intestine decreases rapidly from about 21 per cent of the length of the entire tube in the smallest specimens until a body weight of about 7 gm. is attained and from this weight on to birth the large intestine forms on the average 12.30 per cent of the total length of the digestive tube. The small intestine increases from about 32 per cent in the smallest group of specimens to

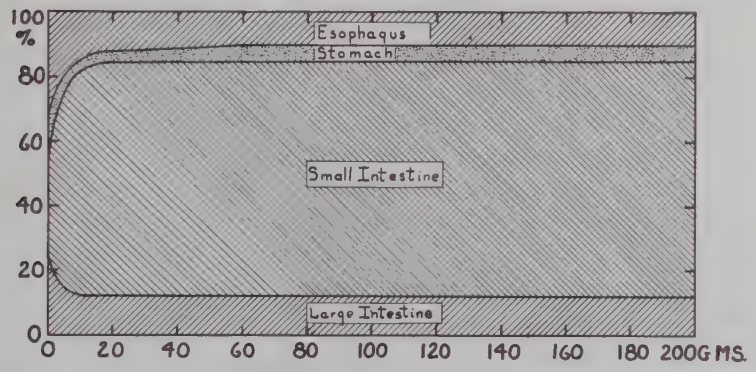

Fig. 5 Each area represents the length of a part of the digestive tube as a percentage of the total length of the tube and plotted on body weight.

an average of 73.03 per cent of the length of the tube between 15 and 200 gm. of body weight. The esophagus, like the large intestine, decreases from about 32 per cent in the smallest specimens to about 12 per cent of the length of the tube in the 20 gm. fetuses. This is followed by a slow decrease to the 60 gm. specimens and from this weight on its average percentage is 9.6 per cent of the length of the entire tube. The stomach decreases from about 13 per cent at first to about 3 per cent in the 10 gm. fetuses and then slowly increases to 5 per cent of the length of the tube in the specimens above 60 gm. of body weight. Thus the small intestine is the only

part which increases rapidly in the early stages of this growth period. A comparison of this figure with figure 2 will show that the relative lengths of the divisions of the tube are established earlier than are the relative weights, and that the lengths are more constant for the later part of the fetal period.

The above percentage lengths were plotted on body length as abscissae and the resulting curves (not shown) did not flat-

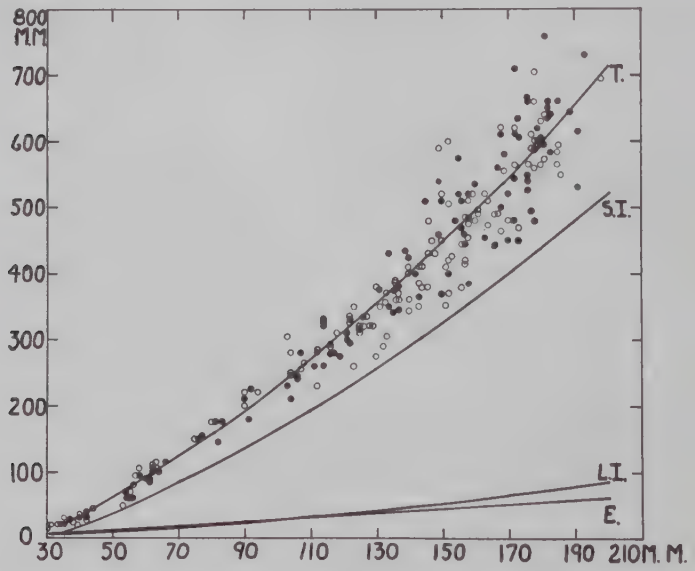

Fig. 6 Lengths of the digestive tube (T.) and of its parts plotted on body length. The dots represent the lengths of the male digestive tubes and the circles, the females. Below this curve are the curves for the lengths of the small intestine (S.I.), large intestine (L.I) and esophagus (E.). The formulae used in constructing these curves are given in the text.

ten out until much later, as was found when the percentage weights of the parts of the tube were plotted on body length rather than body weight. The individual cases of percentage length, like the similar percentages of the weights, were not as closely grouped about the curves when body lengths were used as abscissae as when plotted on body weights.

The lengths of the digestive tube and of its parts (except the stomach) are shown plotted on body length in figure 6.

The individual lengths are shown for the entire tube, the dots representing the males and the circles the females. Only the curves for the three divisions of the tube are shown and the lengths in millimeters are given in the left margin for all of the curves.

The empirical formulae from which these curves were drawn are as follows:

Entire tube (T.), from 35 to 200 mm. of body length,
$Y = 0.01\ (X^3) + 1.83X - 53.3$
Small intestine (S.I.), from 35 to 200 mm. of body length,
$Y = 0.01\ (X^{1.96}) + 1.45X - 60.82$
Large intestine (L.I.), from 30 to 200 mm. of body length,
$Y = 0.001\ (X^{3.07}) + 0.16X - 2.33$
Esophagus (E.), from 30 to 200 mm. of body length,
$Y = 0.33X - 3.90$

In all of these formulae Y represents the length of the digestive tube or of its part measured in millimeters and X represents the body length, or nose-anus length, also measured in millimeters. All of the formulae and the curves fit the newborn kittens as well as the fetuses. The average percentage difference between the observed and the calculated differences for the four curves in this figure is 4.65 per cent. The average for the digestive tube is 5.17 per cent. The lowest average is 2.40 per cent for the length of the esophagus and the largest is 5.63 per cent for the small intestine.

Scammon and Kittelson ('26) give rectilinear curves for the growth in length of the stomach, pylorus, duodenum, appendix and for the large intestine, during the first part of its growth, in the human fetus. They do not give the growth of the esophagus which is the only rectilinear curve found in the lengths of the parts of the tube in the cat fetus. They give a shallow curve for the growth of the small intestine resembling the curve shown in figure 6 for the small intestine of the cat.

It is interesting to note that the curve for the esophagus plotted on body length is the only case of a curve which is rectilinear throughout the entire growth period in the cat fetus. The esophagus has only one function. It grows just enough to maintain the connection between the pharynx and

the stomach and so has a constant ratio to body length. All of the other curves in this figure are concave superiorly. In figure 1, which shows the weights plotted on body weights, all of the curves are likewise concave superiorly. This shows that the growth in length and also in weight of the digestive tube and of its divisions, with the exception of the length of the esophagus, is slower at first and then increases in rate of growth toward the end of the period. Figure 4, which shows the increase in length plotted on body weight, shows that all of the lengths increase more rapidly at first, for all of these curves are convex superiorly. This rapid rate of growth decreases toward the end of this growth period for all of these curves flattened out somewhat. The percentage curves in figures 1 and 4 both rise rapidly at first and then flatten out. This indicates that in both weight and length the entire digestive tube increases more rapidly than does the body weight.

WEIGHT PER UNIT LENGTH

No measurements of the diameters of the tube were made but the weight per unit length was calculated for the entire tube and for the esophagus, the small and the large intestine. The lengths of the stomach were not measured. The weight per millimeter of length was determined for the first group, averaging 0.6 gm. of body weight, and for each 10 gm. increase in body weight, up to and including 190 gm.

The weight in milligrams per millimeter of length for the parts of the tube in the smallest specimens was as follows: esophagus, 0.119; large intestine, 0.148 and the small intestine 0.151 mg. per millimeter. The weights per unit of length at 190 gm. of body weight are not in the same order. They are as follows: esophagus, 5.88; small intestine, 12.14 and for the large intestine 21.94 mg. per millimeter of length. The esophagus is the most slender throughout and its total increase is likewise the least, or but 49.4 times. It has been shown above that its total increase in length and in weight is less than any of the other parts of the tube. It likewise is shown in figure 2, that it forms the smallest percentage weight.

In figure 5 it is shown with a slightly longer percentage value than the stomach.

The small intestine has the next larger total increase in weight per unit of length, or 80.4 times. It has already been shown that the greatest increase in both weight and length is in the small intestine but the increase in length is proportionally much greater than in weight. It is the heaviest division of the tube in the newborn kitten not because of its weight per unit of length but because of its extreme length. It has been shown (fig. 2) that it forms 63 per cent of the total weight of the tube and figure 5 shows that it forms 73 per cent of the total length of the tube.

The greatest total increase per unit of length is found in the large intestine, with a total increase of 148.2 times, or 184 per cent of the increase found for the small intestine. It has been shown above that the large intestine increases in length a little more than the esophagus but less than all of the other divisions of the tube, but its weight increase is second only to that of the small intestine. Hence we may conclude that the greatest growth of the large intestine is in its weight per unit of length.

The weight of the entire tube, computed in the same manner, was found to be 0.250 mg. per millimeter in the smallest specimens, or heavier than any of the three parts given above. This heavy unit weight in these smallest specimens is undoubtedly due to the short and heavy stomach observed in these specimens, and, as well, the relatively large stomach in these specimens. In figure 2 the stomach is shown forming over 52 per cent of the total weight of the tube. The unit weight of the entire tube is but 14.12 mg. per millimeter of length in the oldest specimens. This is but slightly greater than that of the small intestine and much less than for the large intestine. The total increase in weight per unit of length for the entire tract is next to the smallest, or but 56.5 times.

SUMMARY

Empirical formulae are given whereby the weight or the length of the digestive tube or of its divisions may be derived from either the body weight or the body length, within the given limits.

The weights of the tube and of its four subdivisions plotted on body weight or nose-anus length form curves which are concave superiorly showing that they grow at an increasing rate. The lengths of the tube and its parts plotted on nose-anus length are likewise concave superiorly, with the exception of the esophagus which has a constant ratio to the body length and so forms a straight line curve. The same lengths plotted on body weight form curves convex superiorly, or they increase most rapidly at first.

The weight of the entire tube increases from about 1 to a little over 5 per cent of the body weight. The length of the tube increases from about 50 to 375 per cent of the body length.

During this growth period the small and the large intestine increase in relative weight while the stomach and the esophagus decrease. The small intestine alone increases in relative length while all of the other subdivisions decrease. The linear relations are more constant for the larger fetuses and they also become established earlier than do the relative weights.

The total increase in weight for the entire tube and all of its parts is more than the total increase in body weight. The total increases, from least to greatest are as follows: esophagus, stomach, entire tube, large intestine and small intestine. The total increases in the lengths are likewise all greater than the increase in body length and in order from least to greatest they are: esophagus, large intestine, entire tube and small intestine.

The greatest increase in weight per unit of length is found in the large intestine, next in the small intestine, then the entire tube and least in the esophagus. These values were not computed for the stomach.

LITERATURE CITED

JACKSON, C. M. 1909 On the prenatal growth of the human body and the relative growth of the various organs and parts. Am. J. Anat., vol. 9, pp. 119–165.

KEARNEY, HAROLD L. 1914 On the relative growth of the organs and parts of the embryonic and young dogfish (Mustelus canis). Anat. Rec., vol. 8, pp. 271–291.

LATIMER, HOMER B. 1931 The prenatal growth of the cat. II. The growth of the dimensions of the head and trunk. Anat. Rec., vol. 50, pp. 311–332.

———— 1933 The prenatal growth of the cat. III. The growth in length of the two extremities and of their parts. Anat. Rec., vol. 55, pp. 377–394.

LATIMER, HOMER B., AND JOHN M. AIKMAN 1931 The prenatal growth of the cat. I. The growth in weight of the head, trunk, fore limbs, and hind limbs. Anat. Rec., vol. 48, pp. 1–26.

LOWREY, LAWSON G. 1911 Prenatal growth of the pig. Am. J. Anat., vol. 12, pp. 107–138.

SCAMMON, RICHARD E., AND JOHN A. KITTELSON 1926 The growth of the gastro-intestinal tract of the human fetus. Proc. Soc. Exp. Biol. and Medicine, vol. 24, pp. 303–307.

CYTOLOGICAL STUDIES ON THE RAT PITUITARY AFTER INJECTIONS OF PREGNANCY URINE EXTRACT AND PREGNANCY BLOOD SERUM [1]

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Structural changes in the anterior pituitary which result from experimentally induced alterations in other endocrine glands, notably the gonads, have now been described in many species by a large number of authors. Thus, it has been well established that castration and pregnancy alter the structure of the hypophysis. A summary of much of this literature will be found in chapter XIII, Sex and Internal Secretions. More recently, changes in the pituitary associated with the oestrous cycle in the female have been described. A study of pituitary changes becomes increasingly significant when correlated with experiments in which the potency of the gland is assayed. The present paper deals mainly with changes in the anterior pituitaries of male and female rats after the injection of pregnancy urine extracts, and the findings are supplementary to the experimental assay of the gonad-stimulating potency of such glands, previously reported by Leonard ('33).

Meyer, Leonard, Hisaw and Martin ('30, '31, '32), Moore and Price ('32) have demonstrated that the injection of oestrin into normal adult rats reduces the sex-stimulating potency of their pituitaries. Leonard ('33), reasoning that any injection which increased the oestrin output of the ovary should likewise decrease the potency of the anterior pituitary,

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injected a large series of male and female rats with P. U. extracts. These experiments demonstrated a marked decrease in potency of the pituitaries of both male and female rats after P.U. injections. No decrease in potency occurred if the animals were castrated before injections began. These observations confirmed and extended those of Kuschinsky ('31), who, during the course of Leonard's preliminary experiments, independently reported the decrease of pituitary potency in the female rat after P.U. injections. Most of the material to be described here was obtained from the experiments of Leonard, as indicated in his paper. It has been augmented by other pituitaries from the experiments of Dr. P. E. Smith. The author acknowledges these sources of material with thanks.

MATERIALS AND METHODS

A summary of data concerning the rats whose pituitaries were studied cytologically is given in table 1. It is important to know the age of the rat at time of injection, the duration of injections, dosages and the extract used. It will be seen that the female series includes immature as well as mature rats subjected to varying dosages and to varying periods of injection. In one litter the injection was with human pregnancy blood serum. All other rats were treated with Follutein.[2] The male rats, here reported, were all adult when injections began. Either Follutein or Antuitrin S [3] were used, and the dosages ranged much higher, the maximum being a total of 7100 r.u. over a period of 71 days. Ovarian and testes weights, as well as those of the uteri and accessories were recorded, but these data are not included. Ovarian and testes weights were increased, the former sometimes to extreme limits, and the uteri of females and the accessories of the males demonstrated by their enlarged size that the P.U. injections were effective in stimulating the gonads to a great overproduction of their respective sex hormones.

[2] Dr. J. A. Morrell, of E. R. Squibb & Sons, kindly furnished the Follutein.

[3] Dr. Oliver Kamm, of Park, Davis & Co., kindly furnished the Antuitrin S.

The rats were sacrificed by decapitation, their pituitaries promptly removed and placed in a modified Champy, as used in the Nassanov-Kolatchev method. A few minutes after immersion in the fixative, the gland was usually cut through a medium sagittal plane to insure better penetration of the

TABLE 1

Normal and castrated rats injected with P.U. extract

FEMALES					MALES				
Age in days		Treatment			Age in days		Treatment		
At first injection	At autopsy	Extract	Total R.U.[1]	Period, days	At first injection	At autopsy	Extract	Total R.U.	Period, days
21	56	Follutein	62	31	426	445	Follutein	190	19
21	56	Follutein	62	31	405	424	Follutein	190	19
Control	56				Control	445			
					Control	424			
22	56	Follutein	281	34					
22	56	Follutein	281	34	231	251	Follutein	200	20
22	56	Follutein	281	34	Control	251			
22	56	Follutein	281	34					
Control	56				197	244	Antuitrin S	1175	47
					197	257	Antuitrin S	1500	60
249	259	Follutein	100 m.u.	10	197	257	Antuitrin S	1500	60
Control	259				197	257	Antuitrin S	1500	60
23	58	Hum. preg.	17.5 cc.	35	97	172	Antuitrin S	1875	75
23	58	Bl. serum	17.5 cc.	35	97	172	Antuitrin S	1875	75
23	58	Bl. serum	17.5 cc.	35	97	172	Antuitrin S	1875	75
23	58	Bl. serum	17.5 cc.	35	97	172	Antuitrin S	1875	75
Control	58				Control				
Control	58								
					100	171	Antuitrin S	7100	71
					100	171	Antuitrin S	7100	71
					Control	171			
Females castrated at 57 days of age					Control	171			
					Males castrated at 210 days of age				
95	110	Follutein	150	15	247	262	Follutein	150	15
95	110	Follutein	150	15	247	262	Follutein	150	15
95	110	Follutein	150	15	247	262	Follutein	150	15
Control	110				Control	262			
Control	110				Control	262			
Control	110				Control	262			

[1] Unless otherwise indicated.

fixative. The technique in the main followed the procedures previously outlined (Severinghaus, '32). Good results were obtained, however, by following the 20 per cent Altmann's acid fuchsin by a 30-minute treatment with 1 per cent phosphomolybdic acid, and thence, without rinsing, by Masson C for 1 hour, differentiating rapidly in 95 per cent alcohol, clove oil and absolute alcohol. This method is somewhat shorter and gives very satisfactory preparations for the study of cytoplasmic granules and structures. Inasmuch as this study was concerned primarily with changes in the cell itself, no additional technical methods were employed. From the cytological standpoint, a study of the internal organization of the cells themselves in correlation with established changes in the secretion of the gland, constitutes the major interest, for it has a bearing not only upon the specific endocrine problem, but upon the mechanics of cell secretion in general. Particular emphasis, therefore, will again be placed upon the nuclear, Golgi and mitochondrial components of the cell. No attempt has been made to count the cells of a given type in order to establish the proportionate number of such cells. Such data for the rat can be found in the work of Nukariya ('26), Stein ('33), Wolfe ('33). The wide range of variation in their results indicates, perhaps, the difficulties involved in establishing these cell proportions, as well as the variation in the material itself. It is probable that in most instances, a proportional change of sufficient magnitude to be significant could be detected by direct observation of serial sections.

The volume measurements reported in table 2 were obtained by projecting at $\times$ 100 and tracing each third section of the gland, cut serially at 7 μ. These tracings were then measured with a polar planimeter (set 1:1000), the total readings being recorded. Actual volumes of the gland may be obtained by multiplying the total reading by 0.00021. The pituitaries used for these observations were carefully removed intact with the overlying region of the brain, fixed in Bouin, and stained by Masson's triple method.

TABLE 2

Results of Antuitrin S injections into adult male rats

RAT		TREATMENT		WEIGHTS IN GRAMS		RELATIVE POLAR PLANIMETER VALUES		
No.	Weight, grams	R.U.	Days	Accessories	Testes	Pars anterior	Pars intermedia	Total P.A. + P.I.
9505	310	Control	..	2.4	2.17	12306	1662	13968
9507	280	Control	..	2.07	1.95	11764	(1964)[1]	13728
9431	290	Control	..	2.3	1.9	12740	1656	14396
9433	286	25 (½ cc.)	75	4.05	2.5	14207	1876	16083
9434	290	25 (½ cc.)	75	5.2	2.34	14248	1990	16238
9435	280	25 (½ cc.)	75	5.4	3.05	14250	1946	16196
9504	330	100 (1 cc.)	71	4.5	3.2	13912	2241	16153
9436	280	25 (½ cc.)	75	5.4	2.8	Reserved for cytological study		

[1] A massive colloid cyst in the pars intermedia and extensive cellular anastomoses with the pars anterior may be factors in this abnormally large size.

Anterior pituitary of immature injected female

The cells in the anterior pituitary of the immature female rat show marked changes after the animal has been injected with pregnancy urine extract. As typical of these changes, we may describe rats of the first tabulated litter. The rats of this litter received 2 r.u. of Follutein daily for 31 days beginning at the age of 21 days. They were sacrificed 4 days after the last injection at an age of 56 days.

Basophiles. Perhaps the most striking change from normal in these anterior lobes is the almost complete absence of normal basophiles. A considerable search is required, sometimes through several sections, to locate a basophile with normal granulation. They have become degranulated. The cytoplasm outside of the Golgi region is yellowish, at times a fairly vivid orange, and the granular structure has been replaced by one more massive or roughly clumped. In many cells there remain scattered groups of basophilic granules interspersed in the atypical yellow cytoplasm. The yellow color, with the technical method employed, seems due largely to the chromate treatment and subsequent failure of either the acid fuchsin or the aniline blue to stain. Even in extreme overstaining, the aniline blue does not stain.

The Golgi apparatus is typical of the basophile as previously described (Severinghaus, '32, '33), and becomes a very important factor in establishing the identity of these atypical basophiles. It is often greatly hypertrophied and will occupy a large part of the cytoplasm. Frequent small vesicles, which remain clear and unstained, are found, as before, within the Golgi meshwork. The cytoplasm within the Golgi sphere (ring) retains its basophilic character, but shows an accumulation of minute scattered fuchsin granules, which stain exactly as do the mitochondria. The enlargement of the Golgi and its vesicular character in many cells indicates a high secretory activity.

The mitochondria are prominent, and both in size and number exceed those of the normal basophile. They frequently radiate, as earlier described, from the Golgi region into the peripheral cytoplasm, increasing in size at the cell margins. Not infrequently, large accumulations of mitochondrial granules line up along the nucleus. The degranulated basophiles with their abundant mitochondrial granules might readily be confused with sparsely granulated acidophiles by technical methods which did not otherwise differentiate the cells. By the technique here employed, the mitochondria stain in sharp contrast to the acidophilic granules, and the Golgi apparatus, either in negative or positive form, makes the identification of the cells certain.

Among the basophiles at least four characteristic types may be distingushed. These are: 1) Large cells, depleted of basophilic granules, cytoplasm slightly yellowish, Golgi hypertrophied, mitochondria prominent, nucleus palely stained with large nucleolus or with several such bodies. The nucleus is similar to that of a normal basophile in form, size position and staining characteristic. 2) A large degranulated cell with clear cytoplasm, a dark chromatic nucleus, a Golgi body of decreased size, and a scarcity of mitochondria. Such a cell would unquestionably be considered a large chromophobe by observers working with the usual histological techniques. In many of these cells prominent centrioles are found. In

several cells, the second centriole was migrating to the opposite pole of the cell. We have here a cell preparing to divide, and in this reorganization the characteristic cytoplasmic structures of the differentiated secretory cell are disappearing. This type of cell is found much more frequently in the injected adults, where mitotic divisions are also numerous. There are few mitotic divisions in the immature injected female. 3) A degenerative form. The cytoplasm is shrunken and compact and takes a deep basophilic stain. The Golgi ring is atrophied, although distinct, and is the type characteristic of the basophile. The nucleus is pyknotic, stains very densely, but may still have a brilliant fuchsin nucleolus. Areas, in which considerable numbers of these degenerating basophiles mass together, are not uncommon. The cells are angular in outline and frequently elongate. It is possible to find many stages in this degenerative process. 4) A small cell with a prominent Golgi which has hypertrophied to occupy most of the cytoplasm. These cells are very numerous. The Golgi frequently exceeds the nucleus in size. Mitochondria are prominent, and packed into the small margin of cytoplasm which surrounds nucleus and Golgi. The cytoplasm is granular and palely basophilic. These cells frequently dominate the field, and one gets the impression that they are transformed chromophobes which have entered upon an active secretory phase without being able to reach normal basophilic size. As will be pointed out later, the number of typical, clear chromophobes is strikingly reduced.

If we consider these various forms as expressions of functional activity, and that together they constitute the group, basophiles, there can be no doubt but that the total number of basophiles is much greater than in a normal pituitary.

Acidophiles. The acidophiles of the injected immature females are for the most part normal in appearance. A few cells have been noted which are unusually large. The nucleus of such cells is also larger. They may be atypical giant cells which, through sufficient searching, are also to be found in a normal pituitary. Certain cells are in stages of degranula-

tion and show enlarged Golgi and an increased number of mitochondria. However, none of these cell types are present in sufficient number to make a striking contrast to the normal, and any changes, if present, would require careful statistical methods to establish them. As will be seen later, the acidophiles of adult females, injected with P.U. extract and with human pregnancy blood serum do show striking acidophilic changes.

Chromophobes. The chromophobes are normal in type, but definitely diminished in number in the immature injected females. This decrease can be accounted for by their transformation into cells with definite basophilic characteristics. The greatly hypertrophied Golgi of these cells indicates that they are no longer to be classified physiologically as passive, non-secretory cells. However, with technical methods which do not preserve the Golgi and mitochondrial structure, as well as the finer cytoplasmic details, it is certain that these cells would be identified as slightly enlarged chromophobes. Such identification might actually lead to the impression that the injection of P.U. extract is followed by an increase in chromophobes.

Anterior pituitary of the adult injected female

Alterations in the cells of the anterior pituitary after P.U. extract injections occur also in the adult female rat. The changes differ sufficiently from those given for the immature female to require separate description.

Basophiles. A striking feature is again a degranulation of the basophiles, and the appearance of regions of yellowish cytoplasm. The large degranulated type of cell is much more frequent than in the immature female. Except for the granular mitchondria, these cells frequently have little granulation of any kind in the cytoplasm. The nucleus and Golgi apparatus are characteristic of the matured basophile, although the Golgi tends to hypertrophy. There are also many large cells of the type which have a degranulated cytoplasm and a dark-staining chromatic nucleus. In such cells the Golgi apparatus

has undergone a regression and may move even more peripherally in the cytoplasm. The mitochondria are scarce.. As indicated above, these cells frequently have prominent central bodies, and the dense chromatic nucleus is in process of reorganization, preparatory to mitotic division. Mitotic figures are common. This fact, in view of the general paucity of mitoses in the anterior pituitary, is of considerable interest. At division, the greatly reduced Golgi apparatus moves inconspicuously off to a polar margin of the cell, undivided. With its characteristic shrunken ring, however, it still remains the feature by which the dividing cell may be identified.

Many atrophic basophiles are present. The nucleus in these is elongated and pyknotic, staining readily with fuchsin. The cell is shriveled and angular, frequently elongated, and the cytoplasm stains deeply, usually with the aniline blue, but on occasions, with fuchsin. The great variation in the staining character of both the nucleus and cytoplasm is an indication of their abnormality. The Golgi ring, in negative image, especially in Champy preparation, is strikingly clear and leaves no question as to the identity of the cells. In Bouin preparations they are very similar to fibroblasts.

The large number of small basophilic cells to be found in the immature injected female are not present in the injected adult.

Acidophiles. A striking reduction in number, in size, and in affinity for the fuchsin stain takes place in the acidophiles of the adult injected female rat. This is in marked contrast to the male, to be described later. Partially degranulated cells are not uncommon. Large numbers of degenerated acidophiles are present. These are small, irregular, often pointed cells with an elongated nucleus. The cytoplasm stains brilliantly with fuchsin, but has lost its granular structure. The dense chromatic nucleus takes the aniline blue heavily. These degenerate acidophiles resemble closely the degenerate forms described in many species by Trautman ('09). In one pituitary the response of the acidophiles has been such that it is difficult to find even a few which are normal in appearance.

Chromophobes. The chromophobes are more numerous than in the immature injected rats, although one gains the impression that they are nevertheless present in fewer numbers than in the normal. This is a case in point, where statistical methods would need to be employed to establish the fact. The increased number of chromophobes as compared with the immature injected female and the decreased number of small basophilic cells with hypertrophied Golgi indicate a relationship between these two cell types. This is one basis for the inference that the small basophiles have been rapidly derived from the chromophobe reserve in the injected immature female.

Anterior pituitary of the immature female injected with human pregnancy-blood serum

As will be seen by reference to table 1, four immature female rats from a litter of six, where two acted as controls, were injected daily for 35 days with ½ cc. of human pregnancy-blood serum. A total of 17.5 cc. was given. Injections began on the twenty-third day of life. The rats were sacrificed on the last day of injection. The following data from Leonard's records show the response of the ovaries to the injection. The ovarian weights of the injected animals ranged from 0.097 to 0.164 gm.—an average increase over the controls of approximately 650 per cent. Uterine weights were likewise greatly increased. The cells of the anterior pituitary showed marked changes. The most striking feature was the tremendous hypertrophy of the Golgi apparatus in basophiles as well as the acidophiles. This hypertrophy in radical cases led to pronounced fragmentation of the Golgi material into scattered strands, so that the characteristic form was masked, and it became difficult in these instances to use the structure as an identifying feature of the cell. Mitochondria are abundant.

Both types of cells showed forms in stages of pronounced degranulation. Practically no normal granulated basophiles were found. Here, again, there were large numbers of the

small basophiles with the Golgi body occupying almost the entire cytoplasm. The number of clear, normal chromophobes was correspondingly diminished.

Many acidophiles are present, normal both as regards their size and their cytoplasmic components. But there are, in addition to these and to occasional large degranulated forms, numerous large acidophiles in which the Golgi network has completely surrounded the nucleus and branched to completely occupy one end of the cell. Mitochondria are also increased in size and number. Hypertrophied acidophiles of this character have never before appeared in any of my preparations, either in the normal or in a large variety of experimental animals. The atrophic cells, as previously described in the basophiles and the acidophiles, were few in number.

Anterior pituitary of injected castrate female

The pituitaries of castrated females and of castrate females receiving P.U. extract injections were compared. Since Leonard had shown that P.U. extracts are without effect on the pituitary when injected into castrate rats, it was to be expected that no cytological difference would be found in their pituitaries. These expectations were realized, and it is sufficient to state merely that the pituitary of an injected castrate female is cytologically indistinguishable from that of its castrate control. Castration changes have been sufficiently described in previous literature.

Anterior pituitary of adult injected male rat

The anterior pituitary of the adult male rat is altered by injection of P.U. extract. In view of the complete description given above for the female, the male pituitary may be described much more briefly. The response of the male pituitary is, in general, similar in character to that of the female, but the intensity or the degree of the induced changes is much less. Degranulation of the basophiles is again a constant phenomenon, although many normal basophiles are present. The degranulated cells are mostly of normal size, and many

appear to be in earlier stages of granule depletion. The small basophilic cells with yellowish cytoplasm, scattered basophilic granules, and hypertrophied Golgi are much less frequently met with in the male. This same is true of the degenerated basophilic cell with pyknotic elongate nucleus and dense cytoplasm.

The acidophiles of the adult injected male, in contrast to the female, do not show a depletion of their granules or staining ability. On the contrary, they stain more readily, and careful statistical counts would, I believe, show them to be increased in number. The cytoplasmic structures, such as Golgi apparatus, mitochondria and granulation, appear to be normal. The chromophobes show no features of particular cytological interest, although they again give the impression of being present in reduced number as compared with the normal.

One unexpected observation may be of considerable interest. In comparing the pituitaries of animals which had received relatively light dosages over a short period (B819–B859 series or GH3, for example) with those of long, heavily injected rats (GH9433 series), it was found that the cytological changes did not progress in proportion to the amount of injection. On the contrary, the most marked alterations, occurred after moderate injections. With larger dosage, given over an extended period, the pituitaries were more nearly normal. The interest of these findings may be enhanced by preliminary unpublished data in this laboratory which indicate that the interstitial tissue of the testes increases in amount after Antuitrin S injections to a given point from which it then regresses in spite of continued administration of the extract.

Hypertrophy of the pars anterior and the pars intermedia after P.U. injections

It had been suspected during the course of cytological observations on the male pituitary that the pars anterior as well as the pars intermedia were enlarged in the injected rats. Consequently, in two litters of heavily injected rats, the

pituitaries were carefully removed intact within their membranes with the ventral floor of the brain. The tissue was fixed in warmed Bouin and sectioned at 7 μ for reconstruction, as described under Methods. These sections also give opportunity to study the cellular changes under a routine histological fixation and to correlate them with the material here described.

Table 2 shows that a definite and constant enlargement of the pars anterior and intermedia occurs after injections, and that, although the number of cases is small, the data would appear significant. In the case of the cells of the pars intermedia, the usual dark and light basophilic-like cells were present. That they were larger than normal is indicated by nuclear counts in corresponding regions of the gland. The Golgi apparatus is enlarged and in many cells strikingly like the basophilic Golgi in the cells of the anterior lobe. Mitochondria are also similar and present in large numbers. These pars intermedia cells are being studied under various conditions in much greater detail.

Anterior pituitary of injected castrate male

As in the female, described above, the injection of P.U. extract into adult castrated males has no effect upon the cells of the pituitary. The pituitaries show the typical castration changes and the gland of the injected rat cannot be distinguished from the castrate control.

DISCUSSION

A cytological study of the rat pituitary under experimental administration of P.U. extract, as outlined above, was begun with two quite distinct ends in view. The first of these had to do with the alteration of cellular structures during secretory activity, and offered the possibility of additional data which might be added to studies on the thyroid cell, then in progress (Severinghaus, '33). The second and more obvious bearing of such a study would be upon the problem of hormone production by specific cells. Knowing that the gonad-stimulat-

ing potency of the pituitary is being altered, which cells are being affected, and how? An opportunity is offered further to harmonize and interpret the previous descriptions of new cells or cellular changes which have from time to time been reported in the literature. Finally, although they now permit certain speculations concerning the pituitary-gonad interactions, these data when augmented by considerable additional information, now being sought, may be of definite value in the interpretation of that relationship.

It is extremely important to bear in mind that the changes in the pituitary, described above, result after a given series of conditions, and it is not certain that they obtain where these conditions are altered. One needs only to point out the variability in conditions that may exist: age of rat; sex; extract employed and its assayed potency; dosage and period of injections; method of administration; time of autopsy; cytological technique employed. With this in mind, we may briefly discuss these cytological findings.

Cellular mechanism in secretion and release

The experiments of Leonard demonstrated a loss of potency in pituitaries of animals which had received P.U. injections. The cytological examination of such a pituitary shows as its most striking feature a loss of basophilic granulation. These facts taken together are further confirmation that the gonad-stimulating hormone is produced by the basophiles, and that the granulation is the precursor of the secretion. In other words, the granules themselves store in some form the gonad-stimulating principle. We may postulate that the injection of P.U. has a twofold effect upon the basophilic cell, 1) a discharge of the secretion, and, 2) a replacement of its lost store by the cell. The stimulation in initial stages, at least, may call for depletion of the secretory product as rapidly as it can be manufactured by the cell, allowing no storage to occur. Such a gland, while actually in a state of hyper-secretion, would nevertheless show a decreased potency when assayed by implantation.

The cytological findings are in harmony with such a postulation, and with a previous interpretation of cellular modifications in the secretory activity of the thyroid. It was pointed out there (Severinghaus, '33 b) that the mitochondria increase in size and number in the cell during the process of secretion release, and that their swelling seems always to be associated with active secretory interchange between the cell and its environment. Whether this phenomenon involves osmotic changes or alterations in the hydration capacity of lyophilic colloids, the author is unable to state. The same condition obtains in the basophile cell of the pituitary. The normal cell, which may be assumed to be forming and releasing its secretion simultaneously at normal rate, shows a prominence of granular mitochondria. In the castrate, where the basophiles are continuing to secrete, perhaps more actively, Emery ('32), but nevertheless increasing their stored secretion, the mitochandria are much reduced (Severinghaus, '33 a). In the P.U. stimulated cells, where rapid depletion of the secretory granules again occurs, the mitochondria are even more prominent than in the normal.

In the thyroid cells, the Golgi apparatus enlarged markedly after depletion of the follicle-stored secretion, and this enlargement has been interpreted as correlated with the actual production of new secretion to replace the lost store. The actual close association of newly forming colloid with the Golgi meshwork in the experimental sheep thyroid is convincing. The enlargement of the Golgi in the basophilic cells of the rat pituitary after P.U. injections fits into this picture. The Golgi hypertrophies to replace as rapidly as possible its suddenly depleted granulation. If the cell continues to empty under constant stimulation, the Golgi may remain hypertrophied even in the event of complete degranulation and cell shrinkage. Through overactivity the cell may atrophy, become worn out, as do the cells of the thyroid follicle, and we see many such atrophied pituitary cells. It is of interest to note that in those degranulated cells which prepare for cell division, the differentiated secretory structures, such as the

Golgi and mitochondria, undergo marked regression. The cell divides as a simple, non-differentiated cell.

Pituitary changes referable to gonad secretion

There is today an almost overwhelming accumulation of literature dealing with pituitary changes which result from secretion of the gonad. It would be well to bring together briefly such work as has shown histological changes in the pituitary, particularly the rat, in an attempt to harmonize and interpret the data. Engle ('29) was the first to show that, when the gonads were removed, the basophiles increased their stored secretion. Conversely, one might have predicted that an excess of gonadic secretion would deplete the pituitary basophile of its product.

Berblinger ('14) claims to have changed chief cells into pregnancy cells in the young male and virgin female rabbit through injections of placental hormones. Lehmann ('28), injected and fed placental extract to male and to female rats and obtained pregnancy-like pituitaries in every instance. He states further that he can repair castration changes in the male and female pituitaries through the administration of male and female sex hormones, respectively. Zondek and Berblinger ('31) failed to obtain the repair of castration effects in the pituitary by injections of Follikulin, a female sex hormone preparation, although they do state that the number of acidophiles is greatly reduced. Baniecki ('28) produced pregnancy changes in the anterior pituitary of the normal and the castrate female guinea pig by the ovarian hormones, Follikulin and Hogival, but could not find any effect upon the male pituitary. Zondek and Berblinger attempted without success to repeat Baniecki's results in the castrate female rat. Baniecki ('32) repeated his own experiments on the castrate female rat and failed to obtain the so-called pregnancy cells. Both in his latter and in the Zondek and Berblinger experiments injection was begun from 6 weeks to 5 months after castration, whereas in his original experiments which gave positive results on the guinea pig, he began in-

jections directly after castration. Baniecki also reports that with 80 r.u. of Horpan (P.U. extract) daily for 3 weeks he obtained in the adult female rat extensive changes of the chief cells into pregnancy cells, many of the chief cells having greatly increased cytoplasm. The same treatment of male rats showed increased acidophiles but degenerative or atypical chromophobes. He even warns against the use of prolan clinically in the male because of this evident disturbance of the chief cells in their functioning! He reported that the simultaneous daily injection (80 r.u. Horpan and 10 r.u. Fontanon) of P.U. and ovarian hormone for 3 weeks was without effect upon the adult female rat's pituitary. He concludes they are antagonistic in their effect. With human pregnancy-pituitary implants into an adult female, he obtained a great increase in chromophobes. Baniecki feels that the reaction of the implant upon the rat's pituitary is a direct one, since Phillip ('31), has shown the pituitary of pregnancy to be without sex-stimulating potency, i.e., without ability to affect the ovary.

Bachner ('33) has recently reported changes in the female hypophysis following administration of ovarial hormone, and he reviews the pertinent European literature. He obtained typical pregnancy changes in pituitaries of adult female rats after injections of Follikulin (2 cc. daily for 3 weeks). The pregnancy changes consisted primarily of a loss of acidophiles, for certain of these cells become degranulated, and the appearance of large clear cells, derived from the chromophobes. Bachner reports that the loss of acidophilia after oestrin is even greater than in pregnancy.

Desclin ('33 a) injected adult female guinea pigs with P.U. and reported that the pituitary assumed pregnancy characteristics, i.e., voluminous islets of more uniform cellular structure in which the large, feebly-eosin-staining pregnancy cell is the dominating feature. Since the ovaries of the injected females are completely luteinized, Desclin assumes that the corpus luteum effects the hypophyseal changes. He subsequently repeated the observation on the rat ('33 b), using

both castrate and normal adult females. The pituitaries of castrates were not changed by the injections. The pituitary of the injected normal female assumed the structure of the pregnant female, which he describes as a great numerical increase of chromophobes and their size increase to that of the acidophiles. The nucleus is achromatic and vesicular in character and the cytoplasm shows a fine eosinophilic granulation. The proportion of chromophobes to acidophiles is increased.

Friedl ('33) variously injected castrate male and female rats with ovarian preparations (Progynon, Unden) and held the castration changes in the pituitary in check, so long as the injections were maintained. The prevention of pituitary change was more difficult in the male than in the female. He claimed that even placental feeding caused castration cells to become atypical, and many clear cells with pyknotic nuclei appear. Contrary in a way to Baniecki's findings, Friedl states that the simultaneous injection of prolan and Unden will also prevent castration changes, there being here no indication of an antagonistic action between them.

Martins ('31, '33), was able to prevent castration changes in the male rat pituitary by injections of testis hormone, and his experiments indicated that the germinal epithelium elaborated this hormone.

Haterius and Nelson ('32) prevented castration changes in the male rat by replacing the testes with ovary, and they showed further that ovarian implants into a castrated male would remove castration changes which had already set in. Haterius and Charipper ('31) had previously shown that pregnancy changes could be induced in the pituitary of normal female mice by pituitary implants, and further that the substitution of ovary for testes in the male and the subsequent treatment with pituitary implants would produce pregnancy changes in the male mouse. They conclude that the luteinization of the ovary is essential to the development of pregnancy cells in the pituitary. They describe the 'pregnancy cell' as a large ovoid cell with excentric vesicular nucleus, and a

homogeneous acidophilic cytoplasm. These same authors have noted in the rat that basophiles increase markedly during oestrus, especially in cases of experimentally induced continuous oestrus (partial oophorectomy of phenol treated ovaries). A more extensive analysis of pituitary changes related to the cycles has been made by Wolfe and Cleveland ('33), who report that in oestrus, especially late oestrus, the basophiles are only slightly increased, but those of a degranulated type predominate over the normal basophile. In a more recent paper, Wolfe et al. ('33) showed that the injection of placental extracts brought about a slight decrease in number of acidophiles, but the basophiles showed a tendency to increased size and a striking degranulation of their cytoplasm.

The structure of the pituitary of pregnancy in the rat was described by Schenk ('26) and Lehmann ('28) and the majority of European writers follow these descriptions. The histological picture is as follows: 1) A decrease in acidophilia (both number of cells and staining ability); thus, 2) a change from brilliant, contrast staining among the cells to one more faded or monotonish; 3) a cellular picture dominated by large cells with light vesicular nuclei and clear cytoplasm, thought to have arisen from the chromophobes, and by somewhat similar cells, but with chromatic nuclei, thought to be degranulated acidophiles. If this third group of cells is considered as chromophobes, then a chromophobic increase is real, if not, then it is only apparent.

Finally, attention should be called to the careful cytological observations of Urasov ('28), on the pituitary of the pregnant mouse. He describes a basophilic hypertrophy and the presence of new cells, basophilic-like, which arise from the chromophobes.

The setting forth of this rather extensive pertinent literature is important from the standpoint of our present cytological findings. In each case cellular changes have been induced in the pituitary directly through gonadal secretions or by injections which stimulate the gonad to secrete. We

have stated that the injection of P.U. extract causes a degranulation of cells which we can positively identify by their Golgi apparatus as basophiles. These basophiles are rich in mitochondria and have a light, chromatin-scarce nucleus. Certain of them have a heavily-staining nucleus, which in numerous cases demonstrates a preparation for mitotic division. There are, in fewer numbers, large degranulating acidophiles. It is further of interest that many small basophiles with an indicated secretory activity, but without much granulation, are present, and these are believed to arise from the chromophobes. Severinghaus and Rau (in preparation) have noted these same degranulated basophiles, both large and small, in the pituitaries of pregnant rats as a prominent feature. In the writer's opinion, the so-called pregnancy cell, which nearly all investigators have described as an enlarged chromophobe, or a modified acidophile, is, in fact, a type of degranulated basophile as here described, and that the cells produced by P.U. extract or pregnancy blood serum are identical with the 'so-called' pregnancy cells. These cells, being rich in mitochondria, when preserved by the fixatives employed by many above-named workers, would have the appearance of degranulated acidophiles. The actual occasional degranulated acidophiles could not be differentiated from the cells of basophilic origin by most techniques. With this interpretation, only the work of Urasov on the mouse and the recent findings of Wolfe on the rat (placental hormone administration) are in agreement.

The induced changes in the rat pituitary by injections of P.U. extract, while similar in nature, vary in degree in the two sexes, as well as in mature and infantile animals of the same sex, as pointed out in the text. They occur only when the gonads are present, and are thus interpreted to be the result of increased gonadal secretions acting upon the pituitary. Preliminary observations and experiments now in progress, it is hoped, will indicate which of the male and female gonadal hormones are involved.

The one finding constant to all groups of rats injected with P.U. extract is the degranulation of the pituitary basophiles. It will be recalled that all such glands, when implanted, gave evidence of an appreciable decreased gonad-stimulating potency. It is reasonable to assume, therefore, that the basophilic granules represent the source of said potency. These data, when added to those which demonstrated an increased potency associated with basophilic granulation in castration, leave little doubt concerning the gonadotropic function of the basophiles in the anterior pituitary of the rat.

In conclusion, it should be again emphasized that not only are the sex and age of the animal important, but the dosage, potency and length of the period of injections are variable factors which influence results, and they must be taken into account in any description of pituitary changes. The apparent refractory state reached in the male, at which time both the altered gonad and the pituitary return to normal in spite of continued injections of P.U. hormone, is an excellent illustration of this fact.

SUMMARY

1. The injection of P.U. extract, as here employed, causes the following readily detectable changes in the pituitary of the rat:-

a. In immature females. Replacement of normal basophiles by degranulated cells having a cytoplasm of yellowish cast (with the technique employed), large Golgi apparatus, many mitochondria, pale nucleus with prominent nucleolus. Large increase of small, actively secreting basophiles, derived from the chromophobes, which are thereby relatively decreased in number. Certain large dedifferentiating basophiles with deep staining nuclei which are preparing for mitotic division. Acidophiles show very little change.

b. Adult females. Large degranulated basophiles more frequent than in the immature injected rats, but the smaller chromophobe-derived basophile in much fewer numbers. Mitotic divisions numerous. Acidophiles reduced in size, number and staining ability. Many atrophied cells present.

c. Adult males. Degranulation of basophiles, but of lesser degree and extent than in the female. Acidophiles with increased affinity for stain, and probably larger and more numerous than in uninjected males. Chromophobes reduced in number. Pituitaries of long-period, heavily injected rats are more normal in appearance than those subjected to shorter administration.

Results show, therefore, that sex, age, and nature of injection are important factors in any description of pituitary change.

d. Castrate male and females. No pituitary changes result from P.U. injections in castrate animals, indicating that the injection affects the pituitary only through activation of the gonads to increased secretion.

Since degranulation of the basophiles is a constant feature after P.U. injections, and since such pituitaries, when implanted, always show a decreased sex stimulating potency, the conclusion is inescapable that the basophilic cells (basophilic granulation in particular) are the seat of the hormone production. When added to the data of castrate animals, the evidence would seem conclusive.

The degranulated basophile, produced after P.U. extract injections is also present in the pituitary of the pregnant rat and is considered to be the same cell. This 'so-called' pregnancy cell, basophilic in character, is the cell which has most frequently been wrongly identified as a chromophobe or modified acidophile.

Increased mitochondria are associated with active secretory discharge of the cell, and the hypertrophy of the Golgi apparatus with the elaboration of secretion.

LITERATURE CITED

BACHNER, F. 1933 Über typische Veränderungen der weiblichen Rattenhypophyse durch ovarialhormon. Zeit. f. Geburt. u. Gyn., Bd. 106, S. 87.

BANIECKI, H. 1928 Schwangerschaftshypophyse und Ovarialhormon. Arch. f. Gyn., Bd. 134, S. 693.

———— 1932 Hypophysenvorderlappenhormon und Hypophyse. (Experimentelle Untersuchungen an der weissen Ratte). Arch. f. Gyn., Bd. 149, S. 478.

BERBLINGER, W. 1924 Über experimentell hervorgerufene Hypophysenveränderungen. Verhandl. d. deutsch. path. Gesellsch. München, Bd. 17, S. 184.

CHARIPPER, H. A., AND H. O. HATERIUS 1932 The histology of the anterior pituitary of the albino rat in relation to the oestrous cycle. Anat. Rec., vol. 54, p. 15.

DESCLIN, L. 1933 a Influence de la lutéinisation provoquée de l'ovaire sur la structure du lobe antérieur de l'hypophyse chez le cobaye. C. R. Soc. Biol., T. 111, p. 1085.

———— 1933 b Modifications de structure du lobe antérieur de l'hypophyse du rat après injection d'urine de femme enceinte. C. R. Soc. de Biol., T. 113, p. 1526.

EMERY, F. E. 1932 The anterior pituitary sex hormone in the blood and urine of rats. Am. J. Physiol., vol. 101, p. 246.

ENGLE, E. T. 1929 The effect of daily transplants of the anterior lobe from gonadectomized rats on immature test animals. Am. J. Physiol., vol. 88, p. 101.

FRIEDL, F. 1933 Über die durch Hormonzufuhr erziehlte Verhinderung von Kastrationsveränderungen der Hypophyse und Nebenniere bei der weissen Ratte und beim Kaninchen. Zeit. f. Geburt. u. Gyn., Bd. 105, S. 227.

HATERIUS, H. O. 1932 Relation of pregnancy cells in the pituitary of the rat to the reproductive cycle. Anat. Rec., vol. 54, p. 343.

HATERIUS, H. O., AND H. A. CHARIPPER 1931 Experimental studies of the anterior pituitary. II. The occurrence of pregnancy cells in mice following continuous anterior lobe administration. Anat. Rec., vol. 51, p. 85.

HATERIUS, H. O., AND W. O. NELSON 1930 The effect of ovarian implants on castration cell types in the male rat pituitary. Anat. Rec. (Abstract), vol. 47, p. 318.

———— 1932 Experimental studies of the anterior pituitary. I. The influence of ovarian implants on the castration cell type in the male rat pituitary. J. Exp. Zoöl., vol. 61, p. 175.

LEHMANN, J. 1928 Über das Strukturbild der Hypophyse kastrierter und nicht kastrierter Ratten unter dem Einfluss parenteral und enteral Zugeführter Placentarsubstanzen. Vir. Arch. f. path. Anat., Bd. 268, S. 346.

LEONARD, S. L. 1933 Differential effect of prolan in decreasing the potency of the hypophysis in normal and castrate rats. Anat. Rec., vol. 57, p. 45.

LEONARD, S. L., R. K. MEYER AND F. L. HISAW 1930 The effect of oestrin on development of the ovary in immature female rats. Endocrinology, vol. 15, p. 17.

MARTINS, T. 1931 Utilisation des altérations de l'hypophyse consécutives a la castration, comme test d'une hormone testiculaire. Comp. Rend. Soc Biol., T. 108, p. 1080.

———— 1933 Effets de l'ischémie du testicule, après ligature de l'artère spermatique interne, chez le rat. Destruction de l'epithelium séminal, maintien de la genitalia accessoria, altérations du lobe antérieur de l'hypophyse. C. R. Soc. de Biol., T. 114, p. 141.

MEYER, R. K., S. L. LEONARD, F. L. HISAW AND S. J. MARTIN 1930 Effect of oestrin on gonad stimulating power of the hypophysis. Proc. Soc. Exp. Biol. and Med., vol. 27, p. 702.

MEYER, R. K. 1932 The influence of oestrin on the gonad-stimulating complex of the anterior pituitary of castrated male and female rats. Endocrinology, vol. 16, p. 655.

MOORE, C. R., AND T. F. GALLAGHER 1929 On the prevention of castration effects in mammals by testis extract injection. Am. J. Physiol., vol. 89, p. 388.

MOORE, C. R., AND D. PRICE 1932 Gonad hormone functions and the reciprocal influence between gonads and hypophysis, with its bearing on the problem of sex hormone antagonism. Am. J. Anat., vol. 50, p. 13.

NUKARIYA, S. 1926 Über die Bedeutung der Rückenresorption des Spermas (auf Grund von Spermainjektion an Kastraten) und über mikroskopische Veränderungen der Hypophyse an jungkastrierten weissen Ratten. Arch. f. d. ges. Physiol., Bd. 214, S. 697.

PHILIPP, E. 1931 Die biologische Differenzierung der Hypophysenvorderlappenhormone. Zentralbl. f. Gyn., Bd. 55, S. 12.

SCHENK, F. 1926 Über die Veränderungen der Rattenhypophyse in der Gravidität. Zeit. für Konstitutionslehre, Bd. 12, S. 705.

SEVERINGHAUS, A. E. 1932 A cytological technique for the study of the anterior lobe of the hypophysis. Anat. Rec., vol. 53, p. 1.

SEVERINGHAUS, A. E., E. T. ENGLE, AND P. E. SMITH 1932 Anterior pituitary changes referable to the reproductive hormones, and the influence of the thyroid and the adrenals on genital function. Sex and Internal Secretions, ch. XVII. Williams & Wilkins.

———— 1933 a A cytological study of the anterior pituitary of the rat, with special reference to the Golgi apparatus and to cell relationship. Anat. Rec., vol. 57, p. 149.

———— 1933 b Cytological observations on secretion in normal and activated thyroids. Zeits. f. Zellf. u. mikr. Anat., Bd. 19, S. 653.

———— 1934 Changes in hypophysis of adult male and female rats after pregnancy urine extract injections. Proc. Soc. Exp. Biol. and Med., vol. 31, p. 593.

STEIN, S. I. 1933 Experimental studies on the hypophysis cerebri. I. The effects of single pregnancy in the albino rat. Endocrinology, vol. 17, p. 187.

TRAUTMANN, A. 1909 Anatomie und Histologie der Hypophysis cerebri einiger Säuger. Arch. f. mikr. Anat., Bd. 74, S. 311.

URASOV, I. 1928 Die feinere Struktur der Zellen im Vorderlappen der Hypophysis der weissen Maus im Zusammenhange mit der Sekretion und der Schwangerschaft. Arch. Russess. d'Anat., d'Hist. et d'Embryo., vol. 6, p. 167.

WOLFE, J. M., D. PHELPS, AND R. CLEVELAND 1933 Reaction of anterior hypophysis of immature rat to placental hormones. Proc. Soc. Exp. Biol. and Med., vol. 30, p. 1092.

WOLFE, J. M., AND R. CLEVELAND 1933 Cyclic histological variations in the anterior hypophysis of the albino rat. Anat. Rec., vol. 55, p. 233.

———— 1933 Pregnancy changes in the anterior hypophysis of the albino rat. Anat. Rec., vol. 56, p. 33.

ZONDEK, B., AND W. BERBLINGER 1931 Der Einfluss des weiblichen Sexualhormons und der Hypophysenvorderlappenhormone auf die Struktur der Ratten- und Mäuse-hypophyse. Klin. Wchnschr., Bd. 10, S. 1061.

STUDIES ON THE PHYSIOLOGY OF LACTATION

IV. THE ASSAY OF THE LACTOGENIC HORMONE OF THE ANTERIOR HYPOPHYSIS

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The demonstration that some principle of the anterior hypophysis is concerned in the induction of lactation has stimulated numerous attempts on the part of workers interested in lactation studies to investigate the availability of various animals as subjects for the assay of lactogenic extracts. In the early investigations by Stricker and Greuter ('29), Corner ('30), Nelson and Pfiffner ('30, '31), and Turner and Gardner ('31) the common laboratory animals were variously employed, but in a qualitative manner only. With the progress of attempts to purify the active factor it became imperative to employ some method of assay. Gardner and Turner ('33) were the first to introduce a definite assay method. They suggested the use of adult female rabbits whose mammary glands had been developed to the condition of mid-pregnancy by the induction of pseudo-pregnancy. Such animals will lactate as copiously as parturient females when properly stimulated. Gardner and Turner's assay depends upon the injection of the extract under test for 6 consecutive days and an examination of the glands upon the seventh day. The assay rating is judged by the degree of lactation within the glands.

Riddle, Bates, and Dykeshorn ('33) suggested the use of the crop-gland weight of the pigeon or ring-dove, injected for 4 days, as an assay method. They have shown that the crop-gland apparently responds to the same pituitary factor which is active in the induction of lactation. This finding has been confirmed by Turner (personal communication) and the writer.

Lyons and Catchpole ('33), while commenting upon the use of rabbits and pigeons as assay subjects, are of the opinion that the guinea pig is a superior test animal. They suggest the use of adult female guinea pigs which have been spayed during oestrus and which are injected immediately thereafter. The test is judged positive if milk can be expressed by manipulation.

The recently spayed guinea pig would seem to be an untrustworthy animal to employ in the assay of the lactogenic hormone as the writer has shown that adult female guinea pigs will lactate within 30 to 48 hours following removal of the ovaries regardless of whether or not they have been injected with a lactogenic preparation (Nelson, '32, '33). Those findings have been verified and extended. Of 158 females weighing over 400 gm., 137 lactated within 2 days following ovariectomy without having received any further treatment. There appeared to be no constant relation of the oestrous cycle to the type of result obtained as animals in all stages of the cycle at the time of ovariectomy were observed to lactate. Animals weighing less than 400 gm. rarely lactate following ovariectomy. Of forty-six animals of this type only two were observed to lactate. It is very difficult to induce lactation in these younger animals even by the injection of very potent lactogenic preparations as their mammary development is usually not sufficient to allow milk secretion.[1] It is apparent from the above that the use of the recently spayed

[1] The injection of lactogenic pituitary extracts does not appear to induce any demonstrable growth of the mammary glands. The lactogenic hormone seems to be concerned only with the actual secretion of milk and does not directly stimulate proliferation of mammary tissue. This relation has been emphasized by Turner and Gardner, but Lyons and Catchpole ('33 b) state that their extracts induce marked proliferation of the mammary glands, as well as lactation, in spayed virgin rabbits. However, their figures fail to show proliferation of the lobule-alveolar system and only show ducts and end buds engorged with milk. The filling of the ducts with milk, duct lactation, is very apt to mislead the observer into believing that actual growth of the glands has occurred. The writer's early experiments seemed to him to show such growth, but an analysis of the changes induced in the glands by administration of the lactogenic hormone in subsequent experiments on rabbits, rats, dogs, ground-squirrels, and guinea pigs have convinced him that proliferation of mammary tissue occurs only under the influence of the ovarian hormones.

guinea pig in the assay of lactogenic extracts can scarcely be recommended since adult animals usually lactate without further treatment and younger animals do not have sufficient mammary development to permit lactation.

Adult female guinea pigs used without preliminary treatment are much more trustworthy subjects for gauging the presence of the lactation factor in a given extract than the recently spayed animal. The writer has had occasion to use over 300 normal adult females in the evaluation of a considerable number of preparations for the lactogenic factor. In such tests the production of milk, expressible by manipulation, has been used as the index of potency. These experiments have shown that the normal adult female guinea pig may be used to show the presence or absence of the lactogenic factor in a given preparation, and that they may be used to some extent in the quantitative assay of lactogenic extracts. There may be considerable individual variation in the amount of extract required to induce lactation, but if a sufficient number of animals are employed a satisfactory assay may be conducted. However, it is believed that in the assay of the lactogenic hormone it is desirable to use an animal whose mammary glands have undergone proliferation comparable to that present in normally lactating animals. The adult nonpregnant guinea pig, of course, does not possess that development.

Attempts have been made to use male guinea pigs with successful ovarian grafts as test subjects. These animals have enormously proliferated mammary glands and will lactate profusely when injected with pituitary extracts (Nelson, '32, and Nelson and Smelser, '33). However, it has more recently been observed that these animals will lactate also when injected with extracts not containing the lactogenic factor, e.g., pregnancy urine extracts and certain gonadotropic extracts of the anterior hypophysis. Lactation in these instances was always accompanied by luteinization of the ovarian graft and was due, it is believed, to an upset of the gonad-pituitary-mammary gland relationship which seems to be an extremely

delicately adjusted system in these animals. These experiments will be dealt with in a forthcoming publication.

During the course of other experiments (Nelson, '34) it was observed that pregnant guinea pigs from which the uteri and foetuses had been surgically removed did not lactate following the operation. It was further observed that these animals would lactate copiously when potent lactogenic extracts were injected, but would not respond to preparations lacking in the lactation-inducing hormone. The rabbit and pigeon have been used in checking the presence of the lactogenic factor in such extracts. The hysterectomized pregnant guinea pig would thus appear to satisfy the requirements for a standard assay animal and it was decided to employ them in the study of a number of pituitary extracts.

All animals were operated[2] between the fortieth and fifty-fifth days of pregnancy. In ligating the uterine blood vessels prior to excising the uterus, care was taken not to disturb the ovarian arteries as it was demonstrated that if the ovarian blood supply was destroyed a condition of functional castration resulted and lactation occurred. Lactation occurs in hysterectomized pregnant guinea pigs if the ovaries are also removed. Following the hysterectomy, the animals were observed for 4 or 5 days before further treatment was instituted. Injections of the extract under assay was then begun and continued twice daily for 7 days. The test was judged positive if milk could be expressed from the nipples during the test period. Because of the character of the guinea pig mammary gland it is not possible to accurately grade the response by an examination of the glands such as is employed in the rabbit test of Gardner and Turner.

[2] In order to take advantage of the mammary development of pregnancy it is essential that the foetuses be removed. It has been shown (Nelson, '33, '34) that if pregnant guinea pigs are injected with pituitary extracts lactation occurs only after abortion has taken place. The latter occurred regularly in those experiments. Subsequent experiments with more highly purified extracts have shown that abortion need not follow injections of purified preparations, as such animals may continue on to normal parturition despite the occurrence, in some instances, of marked ovarian stimulation. However, in no instance have we ever observed lactation to occur while the pregnancy was maintained.

When potent extracts were injected an abundant secretion of milk occurred. In a number of instances very young guinea pigs were given to and suckled quite successfully by females of this type.

A number of extracts have been evaluated by the use of female guinea pigs hysterectomized during pregnancy. These extracts include alkaline and acid extracts of beef and sheep pituitaries, and several isoelectric precipitation fractions of beef gland extract. In addition, several negative preparations were tested.[3] In all, seventy-eight animals were used in these experiments. The results of a typical test are outlined in table 1. In this instance an alkaline extract of sheep pituitaries was employed.

TABLE 1

Hysterectomized pregnant guinea pigs injected with an alkaline extract of sheep pituitaries

ANIMAL NO.	DAILY INJECTION, FRESH GLAND EQUIVALENTS	RESULT
	mg.	
924	500	Lactation in 36 hours
925	500	Lactation in 42 hours
919	200	Lactation in 36 hours
928	200	Lactation in 48 hours
929	200	Lactation in 42 hours
935	100	No lactation
936	100	Scanty lactation only
939	100	Lactation in 42 hours

Thus it was shown that a daily injection of the equivalent of 200 mg. of fresh gland was sufficient to initiate and maintain lactation in hysterectomized pregnant animals. The equivalent of 500 mg. per day was required to induce lactation in normal adult female guinea pigs. Using the method of Gardner and Turner, it was found that the daily equivalent of 1000 mg. induced a plus four lactation. This extract contained 6 R.U. of gonad-stimulating hormone per gram of fresh gland equivalent. The gonad-stimulating hormone content of the various extracts tested on these animals does not seem

[3] Some of the preparations tested were supplied through the kindness of Dr. Oliver Kamm of Parke, Davis & Co.

to be in any way related to the induction of lactation. Some of the preparations used contained no demonstrable gonad-stimulating hormone when tested on the immature rat and oestrous rabbit. On the other hand, certain extracts containing considerable amounts of gonadotropic hormone did not induce lactation in the hysterectomized pregnant guinea pig. These extracts also were negative when tested for lactogenic hormone on the pseudo-pregnant rabbit.

The pregnant guinea pig hysterectomized during the latter third of pregnancy furnishes an animal that not only has a mammary gland development which is quite uniform, but which also is prepared for lactation. It is obvious that biological assays, which at best are subject to numerous variable factors, should be conducted on subjects as uniform as possible. The hysterectomized pregnant guinea pig appears to be such a subject, and the same is true of the pseudo-pregnant rabbit used in Gardner and Turner's test.

The guinea pig test as outlined above has the obvious disadvantage of requiring considerable time and effort in the preparation of the test animals. For that reason the writer has found the pseudo-pregnant rabbit to be, in many respects, a more convenient subject for the assay of the lactogenic hormone. The method as outlined by Gardner and Turner has been used successfully by the writer and its relative simplicity together with its reliability serve to recommend it for any studies in which the assay of the lactogenic hormone is concerned.

The crop-gland test of Riddle, Bates, and Dykeshorn has the disadvantage of employing a non-mammalian form and in dealing with a response other than milk secretion. However, the fact that the crop-gland appears to respond to the same principle that initiates lactation would seem to render that objection relatively unimportant. The test is very simple, requires no preparation of the subjects, and, since it is judged by the weight of the crop-glands, furnishes a more truly quantitative test than do the other methods. The weight response of the crop-glands of birds on a given dosage is

somewhat variable, but this may be true only in the somewhat heterogeneous class of pigeons usually available, and be less evident in the standardized races of doves and pigeons.

The value of the recently spayed guinea pig, as recommended by Lyons and Catchpole, in the assay of the lactogenic hormone is questionable since lactation usually follows gonadectomy in this species. The explanation for the occurrence of lactation following the removal of ovarian influences has been discussed in another place (Nelson, '34).

SUMMARY

The various methods which have been suggested for the assay of the lactogenic hormone of the hypophysis are discussed.

Particular attention is given to the value of certain types of guinea pigs in assay studies. The female guinea pig hysterectomized during pregnancy is recommended together with the pseudo-pregnant rabbit as standard assay subjects since it is believed that evaluation of the lactogenic hormone should be conducted on subjects with mammary glands prepared for normal lactation.

LITERATURE CITED

CORNER, G. W. 1930 The hormonal control of lactation. Am. J. Physiol., vol. 95, pp. 43–55.

GARDNER, W. U., AND C. W. TURNER 1933 The function, assay, and preparation of galactin, a lactation stimulating hormone of the anterior pituitary. Mo. Agric. Exper. Sta. Res. Bull. no. 196.

LYONS, W. R., AND H. R. CATCHPOLE 1933 a Assay with the guinea pig of the lactogenic hypophyseal hormone. Proc. Soc. Exper. Biol. and Med., vol. 31, pp. 299–301.

———— 1933 b Availability of the rabbit for assay of the hypophyseal lactogenic hormone. Proc. Soc. Exper. Biol. and Med., vol. 31, pp. 305–309.

NELSON, W. O. 1932 The reciprocal hypophyseal-ovarian relationship in the control of mammary gland development and function. Anat. Rec., vol. 54, p. 51.

———— 1933 Reciprocal relationship between ovaries and anterior hypophysis as factor in control of lactation. Proc. Soc. Exper. Biol. and Med., vol. 30, pp. 953–954.

———— 1934 Studies on the physiology of lactation. III. The reciprocal hypophyseal-ovarian relationship as a factor in the control of lactation. Endocrinology, vol. 18, pp. 33–46.

NELSON, W. O., AND J. J. PFIFFNER 1930 An experimental study of the factors concerned in mammary gland growth and in milk secretion. Proc. Soc. Exper. Biol. and Med., vol. 28, pp. 1–2.

———— 1931 Studies on the physiology of lactation. I. The relation of lactation to the ovarian and hypophyseal hormones. Anat. Rec., vol. 51, pp. 51–83.

NELSON, W. O., AND G. K. SMELSER 1933 Studies on the physiology of lactation. II. Lactation in the male guinea pig and its bearing on the corpus luteum problem. Am. J. Physiol., vol. 103, pp. 374–381.

RIDDLE, O., R. W. BATES AND S. W. DYKESHORN 1933 Preparation, identification, and assay of prolactin—a hormone of the anterior pituitary. Am. J. Physiol., vol. 105, pp. 191–216.

STRICKER, P., AND F. GRUETER 1929 Recherches expérimentales sur les fonctions du lobe antérieur de l'hypophyse. Influence des estraits du lobe antérieur sur l'appareil genital de la lapine et sur la montee laiteuse. Press. Medicale, T. 37, pp. 1268–1271.

TURNER, C. W., AND W. U. GARDNER 1931 The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agric. Exper. Sta. Res. Bull. no. 158.

THE WEIGHT OF RAT OVARIES AFTER HYSTERECTOMY

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During the child bearing period, many women submitting to hysterectomy with conservation of both ovaries experience a premature menopause (Sessums and Murphy, '32). The mechanism involved in this sequence is not clear. Two well-known facts, however, point to injury of the ovaries as one link in the chain of events: a) The atrophy of these organs which is associated with the natural menopause, and, b) the premature menopause which follows bilateral oophorectomy.

On the assumption that a disturbance of ovarian function is a necessary sequel of the hysterectomy which is followed by a premature menopause, it can be hypothecated that the operation brings it about in one of two ways: a) By interfering with either the ovarian blood or nerve supply, or both, or, b) by the removal of a hypothetical hormone elaborated in the uterus, and one which is essential for the normal functioning of the ovaries.

The structure and function of animal ovaries conserved at the time that a hysterectomy was performed have been studied by a number of investigators. Removal of the uterus is said to have no effect upon the estrous cycle of the opossum (Hartman, '25), the ferret (Deanesly and Parkes, '32) or the white rat (Long and Evans, '22), and it does not seem to alter the voluntary activity of the latter animal (Durant, '27). Marshall ('22) has observed, a) normal development in the ovary of the rabbit 10 months after hysterectomy and, b) acceptance of the male by the female. He also observed that there was no atrophy in the ovary of the white rat, thus confirming observations of Long and Evans.

On the other hand, Murphy and Sessums ('33) counted follicles in ovaries of rabbits submitted to hysterectomy 6 months or more previously. They found: a) A decrease in the number of primary and secondary follicles; b) more atretic follicles, chiefly primary; c) less stroma; and, d) more atypical interstitial cells than in control ovaries.

The present experiment deals with the effect of hysterectomy upon the weight of the ovaries of the white rat.

MATERIALS AND METHODS

Sixteen operated and sixteen control animals from several colonies formed the basis for the study. All were born within a 30-day period. The exact ages of a few were not known but all ranged between 70 and 100 days, at the time of operation. They were kept under observation for the 2-week period prior to operation and were weighed at 7-day intervals. A litter mate of approximately equal weight was selected for a control when possible. If not available, an animal of approximately the same age and weight was used.

The diet consisted of a constant supply of grain mixed with cod liver oil, and a constant supply of fresh water; green vegetables were fed twice and fresh milk three times a week.

Anesthesia was induced by the intra-peritoneal injection of a watery solution of sodium amytol (10 mg. of the drug per 100 gm. of body weight); the abdominal incision was made in the mid line. When the uterus was removed, care was taken not to disturb the ovaries in any way. Several days after operation, the convalescent animals were placed in the cages with their controls, where they were kept for the remainder of the experiment.

At the end of a 60-day period, each animal and its control was weighed and then killed by chloroform inhalation. The body was measured from the tip of the nose to the middle of the anus.

Both ovaries were freed from their attachments to the body, and cleared of their capsules while lying within the peritoneal cavity. They were weighed together, no attempt being made to alter the moisture of their surfaces.

RESULTS

Essential facts pertaining to the sixteen operated and sixteen control animals are recorded in table 1, where the data are arranged in order of body length. The measurements of the operated animals differ very slightly from those of the

TABLE 1

Data upon weights of ovaries and other body measurements of sixteen white rats Submitted to a hysterectomy 60 days previously, and upon sixteen control animals. Observations arranged according to body length; ovaries weighed in pairs. Note closeness of ovary weights in operated versus control animals

OPERATED					CONTROL				
Identification	Age	Body		Both ovaries	Identification	AGE	Body		Ovaries
		Weight	Length				Weight	Length	
	days	*grams*	*mm.*	*mg.*		*days*	*grams*	*mm.*	*mg.*
JO	?	98	160	43.2	KC	?	100	160	34.8
HO	?	108	162	51.9	HC	?	106	161	50.3
GO	?	108	162	54.3	JC	?	108	163	44.8
MO	?	109	167	39.6	MC	?	119	166	53.8
OO*	134	107	167	45.9	VC*[2]	142	113	167	20.9
KO*	?	112	168	57.7	OC*	?	114	168	45.1
LO	?	119	173	42.9	GC	?	119	170	65.3
AO	130	139	173	45.3	LC	?	125	171	54.2
PO[1]	134	128	174	62.4	WC[5]	142	113	173	30.2
VO[2]	142	130	174	33.4	PC[1]	134	120	173	63.3
ZO[3]	129	125	176	32.5	AC	130	132	175	33.5
XO[4]	126	123	176	36.2	ZC[3]	129	147	178	37.8
WO[5]	142	134	177	33.6	DC	130	142	178	40.3
EO	130	134	180	43.9	EC	130	132	181	28.2
CO	130	151	180	34.5	CC	130	152	183	42.4
DO	130	153	185	43.9	XC[4]	126	154	189	48.9
Average		123.62	172.1	43.82	Average		124.75	172.2	43.36

* = Uterus and one ovary examined microscopically.

[1], [2], etc. = Litter mate with control of same identification.

controls. These differences are well within the range of experimental error, and are in close agreement with measurements given by Donaldson ('24).

One ovary and the uterus from each of two operated and two control animals (marked * in table 1) were examined

microscopically. One pair of operated and control animals (DO and OC, table 1) was selected on account of the closeness of the weights of their ovaries (45.1 and 45.9 mg., respectively); the other pair (KC and VC, table 1) on account of the wide differences in the weights of their ovaries (57.7 and 20.9 mg., respectively). One ovary of each of the four animals was cut in serial sections 6 μ in thickness, and several sections of similar thickness were taken from each uterus. (The histologic findings were confirmed by Dr. George S. de Renyi.)

Microscopic examination showed that the ovaries of nearly identical weights were from animals in the same stage of their estrous cycle, i.e., proestrous stage. Whereas, the animals which supplied the ovaries that differed greatly in weight were in different stages of their cycles. The animal with the smaller ovary was in the proestrous stage, the other animal being in the postestrous phase of the cycle. The presence of many large corpora lutea in the latter ovary explained the greater weight of this organ.

These findings rendered unnecessary further histological study since the slight fluctuations in the weights of the remaining ovaries can be readily explained on the basis that the animals were in different stages of their estrous cycles.

From this study, it appears that hysterectomy performed upon the white rat has no effect upon the weight of conserved ovaries.

LITERATURE CITED

DEANESLY, R., AND A. S. PARKES 1933 Effect of hysterectomy on estrous cycle of ferret. J. Physiol., vol. 78, pp. 80–84.

DONALDSON, H. H. 1924 The rat. Memoirs of The Wistar Institute, no. 6. Philadelphia.

DURANT, E. P. 1927 Studies on vigor. Relation of hysterectomy to voluntary activity in the white rat. Am. J. Physiol., vol. 82, pp. 14–18.

HARTMAN, C. G. 1925 Hysterectomy and the oestrous cycle in the opossum. Am. J. Anat., vol. 35, pp. 25–29.

LONG, J. A., AND H. MCC. EVANS 1922 The oestrous cycle in the rat and its associated phenomena. Memoirs of the University of California, no. 6. Berkeley, Calif.

MARSHALL, F. H. A. 1922 The physiology of reproduction. Longmans, Green & Co., London.

SESSUMS, J. V., AND D. P. MURPHY 1932 Hysterectomy and the artificial menopause. Review of literature, report of ninety-one cases. Surg., Gynec. and Obst., vol. 55, pp. 286–289.

———— 1932 The surgical menopause after hysterectomy with and without ovarian conservation. Surg., Gynec. and Obst., vol. 55, pp. 728–730.

———— 1933 The influence of endometrium upon the rabbit ovary after hysterectomy. Surg., Gynec. and Obst., vol. 56, pp. 600–609.

FURTHER STUDIES ON EPIPHYSEAL UNION IN THE SKELETON OF THE RAT

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Earlier studies on the skeleton of the Wistar albino rat showed that in many long and short bones even of very old animals the epiphyseal cartilages failed to disappear, resulting in a condition of retarded or lapsed epiphyseal union (Dawson, '25, '27, '29). The careful and detailed studies of Todd ('28), in collaboration with many students, on the development of the skeleton of mammals have led him to regard the skeleton as a very delicate indicator of disturbances in the chemical balance of body metabolism. He has concluded that the conditions encountered in the Wistar rats are abnormal and that animals which appear healthy and physiologically normal may still exhibit deficiencies in ossification. It seemed advisable, therefore, to extend the histological observations on the skeleton to other so-called mature rodents to determine how widespread is this phenomenon of lapsed epiphyseal union. The present report deals with the conditions encountered in the Bussey strain of pink-eyed, hooded rats.

Histological studies were made on thirteen animals (seven males and six females) obtained shortly after weaning through the kindness of Prof. Gregory Pincus. They were reared in the laboratory on a uniform, adequate diet and allowed to breed. They were killed at various ages, ranging from 438 to 775 days. In three animals all the points at which lapsed union had been previously observed in the Wistar strain were examined. Since a preliminary study showed that the epiphyseal situation was identical with that of the

Wistar strain, the remaining animals were sampled at random, taking but one or two regions from each for study. All material was fixed in Helly's fluid, decalcified, sectioned in celloidin and stained with Delafield's hematoxylin and eosin.

No detailed description of the findings seems necessary, since in all respects the picture agreed with that found in the Wistar strain. Complete bony union of the epiphyses with the diaphyses was found in the distal end of the humerus, the head of the radius and the distal extremities of the tibia and fibula; but persistent cartilage plates occurred in the head and distal end of the femur, the proximal ends of the tibia and fibula, the distal extremities of the radius and ulna, the olecranon process and the head of the humerus. These features have already been adequately described and illustrated in the three earlier papers. There were slight individual variations but none greater than were encountered in the previous studies.

DISCUSSION

As pointed out by many investigators (Donaldson, '24, and more recently, Evans, '33) the rat grows almost throughout life, but does not do so at anything like a uniform rate. Adulthood is indicated by a sharp change, almost a halt, in the growth rate, with but slow increases in skeletal size after that period. The so-called growth plateau is reached in the case of the female in the fifth month of life and in the male in the seventh month. At this time (Dawson, '25) all the epiphyses of the long bones which unite normally have completed their union with the diaphyses. However, growth of the skeleton does not cease, and the remaining epiphyseal cartilages exhibit varying degrees of activity for some time. Definite histological evidences of lapsed union are not observed until the fifteenth month of life (Dawson, '29). Moreover, the observations of Evans ('33) suggest that, under the influence of the growth hormone of the pituitary gland, activity within the epiphyseal cartilages of the adult rats may be accelerated, although no histological studies have as yet been made on such experimental animals.

That the condition of lapsed epiphyseal union is not restricted to certain inbred strains of rats is indicated by incidental observations by myself on the wild, gray rat and the observations of Shaffer ('10) on the white mouse. In a study of the development of the vertebral columns of several mammals Shaffer illustrates (fig. 16) vertebrae from a year-old white mouse in which persisting cartilage plates are clearly seen.

These observations suggest that hereditary rather than environmental factors may be responsible for the epiphyseal conditions in the rodents. The further elucidation of the problem must await a more extensive histological survey of the epiphyseal regions in such animals, together with the treatment of strains found to exhibit lapsed epiphyseal union with the several endocrine extracts known to influence the process of ossification.

SUMMARY

Earlier studies on the skeleton of the Wistar albino rat showed that in many long and short bones even of very old animals the epiphyseal cartilage failed to disappear. A similar survey has been made of the skeletal conditions in the Bussey strain of pink-eyed, hooded rats ranging in age from 438 to 775 days. In every case the condition of epiphyseal cartilages was found to be identical with that of the Wistar animals, suggesting that this defect in ossification is characteristic of the rat and is due to hereditary rather than environmental factors.

LITERATURE CITED

DAWSON, A. B. 1925 The age order of epiphyseal union in the long bones of the albino rat. Anat. Rec., vol. 31, pp. 1–17.

———— 1927 Further studies on the epiphyses of the albino rat skeleton, with special reference to the vertebral column, ribs, sternum and girdles. Ibid., vol. 34, pp. 351–363.

———— 1929 A histological study of the persisting cartilage plates in retarded or lapsed epiphyseal union in the albino rat. Ibid., vol. 43, pp. 109–129.

DONALDSON, H. H. 1924 The rat; data and reference tables. 2nd ed., Philadelphia.

EVANS, H. M. 1933 Present position of our knowledge of anterior pituitary function. J. Am. Med. Assoc., vol. 101, pp. 425–432.

SHAFFER, J. 1910 Die Rückensaite der Säugetiere nach der Geburt nebst Bemerkungen über den Bau und die Verknöcherung der Wirbel. Sitzgsber. Akad. Wiss. Wien, math-nat. Kl. III, Bd. 119, S. 409–465.

TODD, T. W., AND J. D'ERRICO 1928 Clavicular epiphyses. Am. J. Anat., vol. 41, pp. 25–50.

THE OCCURRENCE OF SCATTERED HAIR-CELLS (?) OTHER THAN TYPICAL NEUROMASTS IN THE LATERAL-LINE CANAL SYSTEM OF THE CATFISH, AMEIURUS NEBULOSUS

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ONE PLATE (SIX FIGURES)

During the course of an examination of the epithelium of the lateral-line canal system of the catfish to determine whether intranuclear crystalloids, such as occur within the cells of the superficial epidermis, were present (Dawson, '33), conspicuous hair-like processes extending into the lumen of the canal were observed. Although the nature and significance of these structures have not been determined, it is felt that a brief record of their occurrence should be made.

The lateral-line canal system in the catfish consists of the lateral-line canal proper, running parallel with the surface of the body, and numerous short, vertical canals leading to the surface and terminating in the so-called pores. The definite lateral-line organs are situated in the canal proper and lie between the vertical canals (Herrick, '01; '03). The structures designated as hair-cells are not localized but are distributed irregularly throughout the entire canal system (figs. 1 and 2) except in the vicinity of the organs; here they are absent. The epithelium of the canals is stratified columnar, consisting of 3 to 4 layers, and resembles that of the general body surface except that no gland cells are present and the intranuclear crystalloids, typical of the superficial cells of the epidermis, cease abruptly at the margins of the pores. The hair-like processes arise from deep-lying cells of the epithelium and reach the surface by passing between

the overlying cells. Accordingly, they frequently are bent one or more times before they emerge into the lumen. This feature often makes it difficult to trace the individual process to the cell of its origin (figs. 3 and 4).

These structures are readily demonstrated by fixation with Helly's fluid followed by Heidenhain's iron hematoxylin or Mallory's phosphotungstic acid hematoxylin—methods which also yield excellent preparations of both neuromasts and taste cells in this animal. Some hesitation was felt about describing them as normal for the catfish; however, since they have been observed in numerous specimens in three successive years, they may be regarded as characteristic of the animal.

The hair-cells are usually ovoid in shape, narrowing slightly at the end from which the processes arise. Their nuclei resemble those of the regular epithelial cells, usually containing a conspicuous nucleolus (fig. 5). A single tapering hair proceeds from each cell. They are usually circular in section but some give evidences of considerable flattening as they emerge into the lumen (fig. 6). The processes are made up of fine, closely packed fibrils which are best distinguished just as the process leaves the body of the cell. Their resemblance to the sensory cells of the crista ampullaris of the mammalian ear is striking (Shambaugh, '32).

Superficially, these scattered hair-cells also resemble the typical neuromasts, but they do not contain the specific cytoplasmic granules which are characteristic of the lateral-line sensory cell and the processes are coarser and longer. However, it was felt that they might be sensory in nature and have the same innervation. After transection of the lateral-line nerve, which resulted in the degeneration of the neuromasts (Brockelbank, '25), these cells remained intact. Recent observations of Rode ('29) indicate that the lateral-line nerve in some teleosts may receive branches from the spinal nerves, but no evidence of this was obtained in the catfish; and the integrity of the hair cells after transection of the lateral-line nerve does not appear to be due to a nerve supply by way of the spinal cord.

A further possibility, that the hair-cells might represent motile elements, was considered, although their location, deep in the epithelium, would not seem to favor this view.

Fresh material was obtained in slices sufficiently thin to permit of examination under oil-immersion. This was done by using a double-edged scalpel, made by cementing two portions of a safety razor-blade, with the cutting edges parallel, into a handle. The bevel of the edges provided sufficient space between the two blades that the integument and lateral-line canal could be cut through without crushing the tissue. The hair-like processes could be readily observed in such preparations. They were highly refractile and non-motile with the exception of a slightly trembling motion probably due to brownian activity. Nothing suggestive of cilary or flagellar activity was noted.

A third possibilty—that the hair-cells might be parasitic protozoa specially oriented within the epithelium and restricted to the canal system—was also considered, but little evidence to support this view could be obtained.

The position, orientation and general morphology of the cells suggests that they are sensory in nature, but it is difficult to ascribe any specific function to them. The recent studies of Smith ('30, '33) with colored solutions show that there is a mechanism of intake and outflow of fluid in the canal system of many fishes. Whether the special cells under consideration act as receptors and play some role in the regulation of the movement of fluids within the canals is problematical.

SUMMARY

The occurrence of scattered hair-cells within the lateral-line canal system of the catfish is reported. Their nature and function remain undetermined. The cells lie deep in the epithelium and send out processes which project for a considerable distance into the lumina of the canals. An examination of fresh material shows the processes to be non-motile.

The cells do not degenerate following transection of the lateral-line nerve and their innervation is unknown. No evidence of anastomoses between the lateral-line nerve and the spinal nerves was obtained. It is suggested that they may be receptors concerned in some way with movements of fluids within the canal system.

LITERATURE CITED

BROCKELBANK, M. C. 1925 Degeneration and regeneration of the lateral-line organs in Ameiurus nebulosus (Les.). J. Exp. Zoöl., vol. 42, p. 293.

DAWSON, A. B. 1933 Intranuclear crystalloids in the superficial epidermal cells of the catfish, Ameiurus nebulosus. Anat. Rec., vol. 56, p. 255.

HERRICK, C. J. 1901 Nerves of siluroid fishes. J. Comp. Neur., vol. 11, p. 177.

——— 1903 On the phylogeny and morphological position of the terminal buds of fishes. Ibid., vol. 13, p. 121.

RODE, P. 1929 Recherches sur l'organe sensoriel latéral des téléostéens. Bull. Biol. France et Belg., T. 63, p. 1.

SHAMBAUGH, G. E. 1932 Cytology of the internal ear. Special Cytology, E. V. Cowdry, ed., vol. 3, p. 1335.

SMITH, G. M. 1930 A mechanism of intake and expulsion of colored fluids by the lateral-line canals as seen experimentally in the goldfish (Carassius auratus). Biol. Bull., vol. 58, p. 313.

——— 1933 The mechanism of intake and outflow of fluids in the lateral-line canal of fishes. Anat. Rec., vol. 56, p. 365.

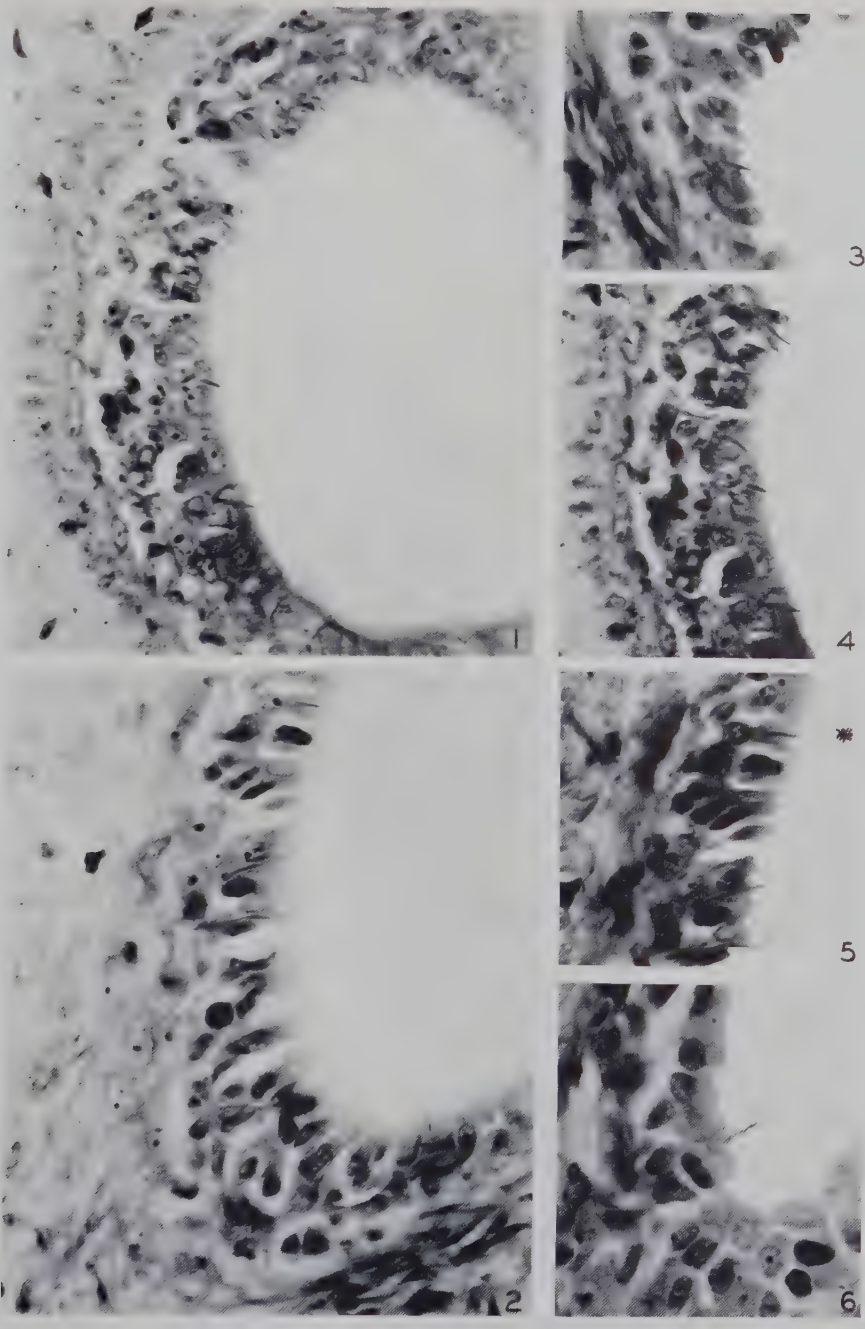
PLATE 1

EXPLANATION OF FIGURES

1 Transverse section of a vertical canal showing the irregular distribution of the hair-cells.

2 Transverse section of the lateral-line proper showing hair-cells; photographed at a deep level to show the extent of the processes within the epithelium.

3 to 6 Small regions, retouched to bring out the extension of the processes into the lumina of the canals. Section of complete cell, indicated by an asterisk, is shown in figure 5.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ON THE PRESENCE, PROPERTIES AND DISTRIBUTION OF THE INTERCELLULAR GROUND SUBSTANCE OF LOOSE CONNECTIVE TISSUE [1]

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ONE PLATE (TWO FIGURES)

The question whether there is or is not an intercellular ground substance in loose connective tissue is still debated. The affirmative is represented in the ideas of Renaut ('03), Laguesse ('14), Maximow ('27), and others, the negative by Nageotte ('22) and his followers.

The former describe lamellae or sheets of amorphous ground substance in which the fibers are enclosed and on the surface of which the cells occur; the lamellae are separated by spaces which are subdivided by continuous strands of fibers or membranes. Nageotte and his followers recognize the lamellae but deny the existence of an amorphous substance. They believe that these lamellae are really made up of a fine feltwork of fibers of varying sizes, the spaces between which may vary in size from the ultramicroscopic to the larger spaces found in the connective tissue, depending on the character of the feltwork. They believe also that these spaces are empty save for tissue fluids.

It is not necessary here to repeat the history of the promulgation of these two views or to review the work of the supporters of these theories. These are well reviewed by Laguesse ('14), Maximow ('27) and Nageotte ('22).

[1] This investigation was accomplished with the aid of the Henry Strong Denison Medical Research Foundation.

I wish here to acknowledge the help and suggestions of Drs. R. R. Bensley, A. A. Maximow, A. B. Hastings, L. H. Hyman, P. Kyes and G. W. Bartelmez.

The results of the investigations to be described here seem to indicate the presence of an amorphous substance and they indicate some of its properties. The presence of the amorphous substance is demonstrated in four ways:

1. Observation of fresh material

It is generally supposed that in the study of subcutaneous tissue by Ranvier's 'bulla' method no amorphous substance may be seen. This is undoubtedly due to the difficulty of studying fresh connective tissue under ordinary conditions. One of three conditions must exist, either: there is no amorphous substance, or the water or salt solution dissolves the amorphous substance, or it has the same refractive index as the medium in which the tissue is mounted. By using a medium with a different refractive index from that of the tissue fluids, this difficulty may be in part overcome.

Bullae were therefore made with homogeneous serum in guinea pigs and rabbits. Such a serum would not be likely to dissolve any amorphous substance if present, and the refractive indices of the solution ordinarily used for the 'bulla' and serum are quite different.

When treated in this way and the bulla excised, the tissue appears under the microscope as usual: a loose network of collagenic and elastic fibers, save that at the cut edge of the tissue, in the intervals between fibers, the edge of the amorphous substance is seen as a fine line which appears and disappears, on focusing, without giving the refractive phenomenon of double lines which even the finest fibers present on focusing up and down. This amorphous substance, when followed, loses itself in continuity with a fiber. Thus, the ground substance is best seen in this material in places where it is put under a strain between two fibers, at the cut edge of the tissue.

2. Bullae made with a suspension of paramoecia

A culture of paramoecia was gradually adapted to a 0.6 to 0.8 per cent salt solution. This culture was injected subcutaneously into a guinea pig; the bulla was excised and treated as a whole mount in that a coverslip was supported on bits of broken glass over the tissue, and the preparation was watched for a considerable time. This experiment was undertaken in the hope that the organisms would reveal by their movements barrier substances which would prevent their escape at points where otherwise there might be no barrier.

The paramoecia were seen to react in a peculiar manner: they were very active, swam vigorously back and forth, and suddenly rebounded without coming in contact with any visible structure; some showed constrictions in two or three places which were not produced by fibers. Eventually all died in the places in which they were observed. None escaped into the surrounding fluid from the bulla.

According to Doctor Hyman, paramoecia in viscid substances such as gelatin show constrictions and distorted forms, and when placed in a drop of acid they rebound in the manner described above. It would seem that if the subcutaneous tissue consisted only of fibers some of the paramoecia would have escaped into the fluid surrounding the bulla.

3. Repetition of Renaut's ('03) experiment

Renaut attempted to demonstrate the amorphous substance by teasing an excised piece of subcutaneous connective tissue on a slide, fixing it, making an x-incision in it after staining the preparation with aniline blue. Here he saw a homogeneous blue background with the fibers staining a darker blue, except for the x-mark which was colorless. The blue background he interprets as the ground substance. There are two criticisms of this interpretation. The blue background may be an optical illusion due to the network of blue fibers acting like a color filter, or it may be due to the colloidal dye which is distributed on the slide and which has been scratched off by the x-incision.

This experiment was repeated by making the x-incisions before fixing, after fixation before staining, and after fixation and staining. In every case the x-incision was colorless but the border of the x-incision was sharp and clearly differentiated and showed a homogeneous blue-stained substance between the cut ends of the fibers. This result seems to indicate the existence of a definite intercellular substance.

4. Differential staining of the amorphous substance

One convenient source of connective tissue in large enough quantities to handle with ease is afforded by the fibrosis of the pancreas of the guinea pig which results in 14 to 19 days after the ligation of the pancreatic duct.

If pieces of such connective tissue are fixed for 48 hours in a solution of nine parts Zenker stock (without acetic acid) and one part redistilled neutral formalin, subsequently embedded in paraffin, sectioned and stained with a 1 per cent solution of toluidine blue and examined in water, the ground substance will be seen to be pink while the fibers and cells stain blue. The differential staining is due to the metachromatic properties of this dye. As in the case of thionin applied to mucin (Lee, '28), the pink stain given to the ground substance by toluidine blue can be changed into blue by alcohol and back to pink again by water. Therefore, these sections must be studied in water or be treated with a solution of equal parts of 5 per cent ammonium molybdate and 1 per cent potassium ferrocyanide, which protects the metachromatic stain against the reversing effect of the alcohol to some extent, and they can be cleared in xylol and mounted in balsam. These preparations fade in time but may be used for a year or so.

Other sources from which connective tissue was obtained and studied by this method were: the subcutaneous tissue of adult animals, subcutaneous tissue of the human skin from areas showing fibrosis due to carcinomatosis, sections of uterine mucous membrane from various stages of the menstrual cycle, and from the cock's comb. The same general results were obtained in tissue from each of these sources.

Other methods were used to demonstrate the ground substance and these with the experiments described above demonstrate some of the properties of the ground substance. These may be reported under eight headings:

1. Appearance

In pieces of fibrosed pancreas excised 14 days after duct ligation, mounted in fresh homogeneous blood serum, and studied under the microscope with the light cut down, the ground substance appears as a continuous, finely granular, transparent substance in which the fibers and connective tissue cells are embedded.

2. The refractive index

It has been shown that the refractive index of the ground substance is about the same as that of water and isotonic salt solution but is different from that of blood serum or a solution of cane sugar. Of course the refractive index of the serum is highly variable depending on the amounts of serum albumen and globulin present. The refractive index of the sugar solution also varies with its concentration. The refractive indices of one series of solutions were determined with the aid of Doctor Hastings and found to be:

Temperature 2.8° (room temperature)

Solution	*Refractive index*
Distilled water,	1.3330
Isotonic salt solution (0.85 per cent),	1.3343
Fresh rabbit's serum	1.3483
50 per cent cane sugar,	1.3869

Since the ground substance is invisible when the connective tissue is mounted in water or salt solution but is visible when the medium is homogeneous serum or sugar solution, its refractive index must lie between 1.33 and 1.34.

3. Consistency

In watching a piece of connective tissue cut from a bulla made with homogeneous blood serum, the edge of the amor-

phous substance at the cut surface is seen to change constantly. It recedes toward the main mass of the tissue until it finally envelops the outermost fibers and thus becomes invisible. This shows that the substance is elastic and tends to retract when cut. Pieces of tissue relatively rich in ground substance appear and feel gelatinous, indicating the jelly-like consistency of the amorphous substance.

4. Extraction with NaCl and half-saturated lime water

By using a modification of the methods used by Chittenden, Richards and Gies (1896 and '02) for extracting mucoids from tendons, the ground substance may be extracted.

A piece of rabbit's omentum was fixed on the slide and stained according to Renaut's method save that Mallory II was used in place of the acid methyl blue. The typical omentum picture was observed: amorphous blue staining material containing fibers, connective tissue cells, aggregates of lymph tissue, etc., interrupted by fat vacuoles and covered with mesothelial cells.

Another piece of the same omentum was fixed on the slide in the same way by von Lenhossék's solution, treated with 10 per cent NaCl solution and placed under a dripping stream of half-saturated solution of lime water for 24 hours. It was washed with water and stained like the control piece. In this preparation all the cellular elements were gone, leaving only the connective tissue fibers and a homogeneous blue staining substance. The latter was continuous except where interrupted by holes made previous to staining, and the boundaries of the tissue were distinct.

The rest of the omentum was removed and extracted with 10 per cent NaCl. Then it was put into half-saturated lime water (4 cc. for every milligram of fresh tissue) for 24 hours at room temperature. It was then washed and pieces were excised and put on the slide, fixed with von Lenhossék's solution, stained with Mallory II, and studied. The cellular elements were all gone. Only the collagenic fibers remained and these were strings of droplets in some places at the pe-

riphery. No amorphous substance was apparent save in some areas along the fibers. There were no distinct boundaries of the tissue even at the x-incisions made before and after staining. In other pieces where the extraction was not so complete, the fibers and some homogeneous blue substance remained, but the latter was very irregularly distributed.

From this experiment it would seem that the ground substance, like the mucins of the connective tissue fibers is extractable by 10 per cent NaCl and half-saturated lime water; that fixation with von Lenhossék's solution renders it more resistant to extraction; that the cells of the connective tissue are the first to be extracted, the ground substance next and the fibers are the last to be extracted.

5. Pancreatin digestion

The amorphous substance is digestible with pancreatin. Digestion experiments with Merck's pancreatin after the methods[2] employed by Mall ('00), Spalteholz, Kyes ('01), Hoehl, Clark and Flint ('02) were undertaken on pieces of fibrosed pancreas following duct ligation. These pieces were fixed for 24 hours in Carnoy's fluid, washed and digested in pancreatin and alkali (0.5 per cent $NaHCO_3$) for 24 hours at 24°C. They were then washed, dehydrated, cleared, embedded, sectioned and stained with Mallory's connective tissue stain. Other pieces were fixed, embedded, sectioned and mounted on slides without albumen before digestion. After removing the paraffin these preparations were digested with pancreatin at 33 to 35°C. for 24 hours. They were then washed and stained with Mallory's connective tissue stain.

Both series showed the same picture with slight quantitative variations depending on the degree of digestion. After pancreatin digestion the fibers remained but with an occasional fibroblast and rarely a homogeneous blue mass, in cases where digestion was not quite complete.

[2] Ewald and Kühne were the first to use tryptic digestion in the study of tissues. Verhandl. d. naturh.-med. Ver. zu Heidelb., Bd. 1.

6. Pepsin digestion

Simultaneously with the pancreatin digestion, pepsin digestion experiments were carried out on pieces of the same tissues with the use of Merck's pepsin and 3.3 per mille HCl. The same procedure was used as in the pancreatic digestion. The resulting pictures were just the reverse of those seen after the pancreatin digestion. Here the fibers had disappeared almost entirely and there remained only a blue mass, with some blue precipitates and an occasional fiber. (Some of the pancreatic cells remained in sections from the pieces digested before embedding.)

The interpretation of the peptic digestion experiments is not as simple as that of the pancreatic because of the possibility that some of the products of the digestion of the fibers may constitute a part of the residue. However, it does appear that the ground substance is more resistant to peptic digestion than are the fibers.

This is in accordance with Mall's findings for exoplasm ('01):

> When the main mass of the syncytium is formed of exoplasm it is digestible in pancreatin and bicarbonate of soda. . . . . The action of pepsin and hydrochloric acid upon the connective tissue syncytium appears to be the opposite of that of pancreatin. . . . . When the digestion is not complete the white fibrous tissue is dissolved first, then the cartilage and then the syncytium. . . . . The connective tissue syncytium is more resistant toward the action of pepsin than is the white fibrous tissue.

7. Staining properties

Besides the various connective stains previously used for the staining of the amorphous substance (Renaut, Laguesse, Mallory, et al.) it can be stained differentially with toluidine blue as described above.

This stain is not a microchemical test for the ground substance of connective tissue or for mucins, although it does, like thionin, stain mucins metachromatically. By treating

sections with dilute acids or alkalis, the physical state of the structures in the sections may be modified so that everything stains metachromatically with this dye. Moreover, if the dye is used in too concentrated a solution or for too long a period, the differential staining of the connective tissue is lost and all the elements save the nuclei may be stained metachromatically. But with uniform methods of fixation as described above, this dye gives us just a method of differentially staining the amorphous substance and the fibers of the connective tissue.

8. Affinity for copper salts

The ground substance has an affinity for copper salts. It has been suggested that the intercellular substance of connective tissue, like that of cartilage, may contain derivatives of chondroitin sulphuric acid. Schmiedeberg (1891) states that chondroitin sulphuric acid obtained from cartilage forms compounds with copper and iron. If this be the case, it might be expected that the ground substance could be mordanted with copper salts. Preparations of adult subcutaneous tissue, teased thin and dried on a slide, mordanted in copper sulphate for 15 to 30 or more minutes and stained with colorless hematoxylin show a blue homogeneous substance which envelops and extends along the collagenic fibers. This treatment does not produce general staining of the glass slide, and tissue spaces remain unstained.

DISTRIBUTION

That there is a difference between the intercellular substance of newly formed and adult connective tissue is apparent. This difference is chiefly in its consistency and distribution. The transition is best seen in the stages of fibrosis of the guinea pig pancreas following duct ligation. At first the pancreas becomes edematous and the stroma is distended and its elements are spread apart by this edema. At this stage the fluid filling the spaces does not stain metachromatically with toluidine blue.

During the first 10 days to 2 weeks there is a proliferation of fibroblasts, and then the pancreas becomes more gelatinous and of a more viscid consistency. Sections from this stage stained in toluidine blue show a metachromatically staining ground substance completely filling the spaces between the cells and fibers with practically no tissue spaces. Sections impregnated with silver by the Foote technique show new reticular fibers running through this ground substance.

At 19 days after ligation the pancreas is much firmer and more fibrous. Sections from this stage, stained with toluidine blue show ground substance in which spaces begin to appear. These spaces do not stain metachromatically, are clear, and look like newly formed tissue spaces. In the ground substance reticular fibers are seen, in sections impregnated with silver. These fibers tend to group themselves and some are continuous with collagenic fibers, which stain deep blue with Mallory's connective tissue stain. Around these groups of reticular fibers the metachromatic staining of the ground substance is deeper. Where glands remain in the tissue the fibers appear more abundant around these glands and are associated to form a thick basement membrane which also stains a deeper pink due to the greater amount of ground substance here.

Similar results are found in the cyclic changes of the stroma of the human uterine mucous membrane. A series of sections of uterine mucous membrane at various stages of the menstrual cycle, from the Culbertson collection of specimens, were stained with toluidine blue and with silver and studied.[3]

[3] The material studied was from the following specimens:

cc. 302	2nd day	menstruating
cc. 239	2nd day	menstruating
cc. 282	4th day	repair
cc. 313	5th day	repair
cc. 251	11th day	proliferation
cc. 283	11th day	proliferation
cc. 262	30th day	late proliferation
cc. 261	46th day	late proliferation
cc. 233	33rd day	progravid
cc. 1261		early gravid

Some of these specimens have been described and analyzed by O'Leary ('29) and Bartelmez ('31).

Following menstruation there is a period of repair when, in the stroma of the mucous membrane there is a slight edema followed by a proliferation of fibroblasts. At the first stage (of slight edema) there is practically no metachromatically staining ground substance, and there are relatively few reticular fibers in the active area. During the period of proliferation the stroma may increase in thickness almost twice over and there is a continuous intercellular metachromatically staining ground substance in which new formed reticular fibers are present. When the stroma has practically completed its growth (except in a narrow margin at the periphery of the mucous membrane), practically no metachromatically staining substance is seen, except in this region and in the intima and media of the blood vessels, but reticular fibers are abundant.

In the premenstrual period the mucous membrane becomes edematous, the reticular fibers and cells are spread apart by the edema fluid and the section presents essentially the same picture as is seen immediately following ligation of the pancreatic duct. If menstruation does not occur or if pregnancy intervenes, the edema fluid is changed into or replaced by metachromatically staining ground substance around the decidua-like cells, and the latter appear like young cartilage cells surrounded by their new-formed matrix. In this newly formed ground substance new reticular fibers appear and the sequence of events is repeated.

This distribution of the ground substance seems to be characteristic of embryonic and reticular tissue: it presents a continuous intercellular, jelly-like substance, in which reticular fibers appear, and which is remarkably free of tissue spaces. This distribution has been observed in the study of umbilical cord, the intima and media of blood vessels, the connective tissues of lower vertebrates, mucous membrane of the stomach, early fibrosis in carcinoma of the skin, and in various other tissues.

In the late stages of fibrosis of the pancreas, sections show a condition more like that which exists in adult mammalian

subcutaneous tissue. In the former, tissue spaces are conspicuous as are the collagenic fibers, the ground substance appears to be reduced or modified and is found in the neighborhood of the collagenic fibers. In adult subcutaneous connective tissue the ground substance is interrupted by large tissue spaces and tends to be massed around the fibers, so that the tissue has lost much of its sheet-like homogeneous appearance and resembles rather a feltwork.

Miss Hardesty ('31) in her work on the response of the fowl's comb to sex hormones, has demonstrated two such types of distribution of the ground substance side by side in the adult cock's comb: in the intermediate zone, reticular tissue with abundant, continuous metachromatically staining intercellular substance, and in the peripheral zone the adult type of subcutaneous tissue with a less evenly distributed ground substance, dense collagenic fibers and tissue spaces.

The Clarks ('33) in studying the growth of lymphatics by the transparent chamber method in the rabbit's ear and in the transparent tail of the amphibian larva, describe the presence of a continuous jelly-like intercellular substance. They distinguish this from tissue fluid by its consistency and by the absence of brownian movement in the ground substance as compared with transudates and exudates. They believe that normally there are no tissue spaces in the strict sense. In the transparent chamber, even after 3 months, with the formation of adult mammalian collagenic tissue, they find no tissue fluid by the criterion of brownian movement. They also describe the phenomenon of the change of edema fluid into, or the replacement of it by the jelly-like intercellular substance.

These observations of the Clarkes are in full accord with the results of the experiments and studies described here on the presence, formation and distribution of the ground substance, with the exception of the later formation and occurrence of tissue spaces. However, the mechanics of growth of connective tissue may be different in transparent chambers and in freely growing tissue, and may preclude the formation of new tissue spaces in developing collagenic tissue in the transparent chambers.

DISCUSSION

The reactions of the paramoecia in bullae of subcutaneous tissue suggest the presence of a viscid substance, possibly acid in reaction. The metachromatic staining of the intercellular substance suggests a resemblance to mucins. The fact that this substance can be extracted with lime water suggests that it may be mucoid, comparable with the mucoids in tendons. Its affinity for copper salts suggests that it may contain a derivative of chondroitin sulphuric acid; although this is not a very strong suggestion as copper and iron salts have a capacity for mordanting proteins of various sorts. However, the staining of the ground substance is deeper than that of protoplasm after mordanting in copper. From the results obtained by pancreatic and peptic digestion of connective tissue taken from a pancreas in a state of early fibrosis, it appears that the continuous ground substance is identical with the exoplasm of Mall.

The origin of this ground substance has not been demonstrated as yet. Hardesty ('31) states that it is secreted by the fibroblasts but gives no objective evidence for this. The sequence of events in fibrosis of the pancreas and in the growth of the stroma during the cyclic changes of the human uterine membrane, namely, the proliferation of fibroblasts, the appearance of a continuous ground substance around the connective tissue cells, the formation of reticular fibers in this ground substance, suggests that it may well be derived from these cells.

From the fact that the ground substance can be extracted with lime water as can the mucoids of tendons, and from conconsideration of the relation of the ground substance to collagenic fibers, it seems possible that the cement substance of the collagenic fiber may be a modified form of this ground substance. The chemical and physical differences between reticular and collagenic tissue may be due to this difference in the relation of the fibers to the ground substance.

Some evidence of this is afforded by results obtained with the silver impregnation technique of Foote. Usually under

standard conditions, only the reticular fibers are impregnated. If, however, the period of impregnation is varied, the silver is deposited first on the ground substance, next on the reticular fibers, and, finally, after an extensive period of impregnation, on the collagenic fibers. This same phenomenon has been described by Owens and Bensley ('31) for reduced osmium, which may be made to deposit on protoplasm, Nissl bodies, on the periphery of or within the Golgi apparatus, by varying the conditions of impregnation. Thus, the ground substance, like a protective colloid, may serve to inhibit the deposition of reduced silver on the collagenic fiber.

The difference in reactions of the reticular and collagenic fiber with dilute acetic acid may be explained also on the basis of this relation. Undoubtedly, the ground substance is in a complex colloidal state. It is well known that weak acids increase the capacity of lyophilic colloids to absorb water. The hydration of the ground or cement substance due to the acetic acid may render its refractive index the same as that of the fibrillae of the collagenic fiber and thereby make them both invisible. That reticular fibers do not react in this way may be explained by the fact that the ground substance enveloping them is not in the nature of a cement substance, i.e., there is not so much of it and it is not as condensed. Therefore, its hydration capacity is not increased to such a great extent and the refractive index is not appreciably changed.

SUMMARY AND CONCLUSIONS

The presence of the ground substance is demonstrated by:

1. Making it visible in fresh preparations by mounting the tissue in a medium of a different refractive index.
2. The reactions of paramoecia injected into a subcutaneous bulla.
3. A confirmation of Renaut's experiment.
4. Selective stains.

The ground substance has certain characteristic properties:

1. Its appearance.
2. Its refractive index.

3. Its consistency.

4. It may be extracted by 10 per cent salt solution and half-saturated lime water.

5. It is digested with pancreatin.

6. It is resistant to pepsin digestion.

7. It may be made to stain metachromatically with toluidine blue.

8. It has an affinity for copper salts.

Since the ground substance varies with the physiological age and development of the connective tissue, it is different in different localities. Thus, in embryonic and undifferentiated tissues, such as reticular tissue, the ground substance remains in its young, viscid, continuous form. In adult subcutaneous tissue it is less abundant, interrupted by tissue spaces, and chiefly encloses the connective tissue fibers. This variation in the consistency and distribution of the ground substance may account in part for the discrepancies in the two major views as to the presence of such a substance.

LITERATURE CITED

Bartelmez, G. W. 1931 The human uterine mucous membrane during menstruation. Am. J. of Ob. and Gyne., St. Louis, vol. 21, p. 623.

Chittenden, R. H., and W. J. Gies 1896 The mucin of white fibrous connective tissue. J. Exp. Med., vol. 1, p. 186.

Clark, E. R., and E. L. Clark 1933 Further observations on living lymphatic vessels in the transparent chamber in the rabbit's ear. Am. J. Anat., vol. 52, p. 273.

Flint, J. M. 1902 A new method for the demonstration of the framework of organs. Johns Hopkins Hosp. Bull., vol. 13, p. 48.

Foote, N. C. 1924 A technic for demonstrating reticulum fibers in Zenker-fixed paraffin sections. J. Lab. and Clin. Med., St. Louis, vol. 9, p. 777.

Hardesty, Mary 1931 The structural basis for the response of the comb of the brown leghorn fowl to the sex hormones. Am. J. Anat., vol. 47, p. 277.

Kyes, P. 1901 The intralobular framework of the human spleen. Am. J. Anat., vol. 1, p. 37.

LaGuesse, E. 1914 La structure lamelleuse du tissu conjunctif lache chez la torpille. Arch. d'anat. mic., T. 16, p. 67.

Lee, A. B. 1849–1927 The microtomist's Vade-mecum, 9th ed., p. 136, par. 214. P. Blakiston's Son & Co., Philadelphia. 1928.

Mall, F. P. 1900 Reticulated tissue, and its relation to the connective tissue fibrils. Johns Hopkins Hosp. Reports, vol. 1, p. 171.

———— 1901 On the development of the connective tissue from the connective tissue syncytium. Am. J. Anat., vol. 1, p. 329.

MAXIMOW, A. A. 1927 Bindegewebe und blutbildende Gewebe. Handbuch der mikroskopischen Anatomie des Menschen, Bd. 2, S. 247.

NAGEOTTE, J. 1922 1. Il n'y a pas de substance amorphe dans la trame conjonctive. Cpt. rend. des séances de la soc. de biol., T. 87, p. 147.

———— 1922 2. La boule d'edème de Ranvier et la disposition de la trame dans le tissu conjonctif souscutané. Ibid., p. 439.

O'LEARY, J. L. 1929 Form changes in the human uterine gland during the menstrual cycle and in early pregnancy. Am. J. Anat., vol. 43, p. 289.

OWENS, HELEN B., AND R. R. BENSLEY 1931 On osmic acid as a microchemical reagent, with special reference to the reticular apparatus of Golgi. Am. J. Anat., vol. 44, p. 79.

RENAUT, M. J. 1903 La substance fondamentale continue du tissu conjunctif lache. Cpt. rend. des séances de la soc. de biol., T. 55, p. 1620.

RICHARDS, C. N., AND W. J. GIES 1902 Chemical studies of elastin, mucoid and other proteids in elastic tissue with some notes on ligament extractives. Am. J. Phys., vol. 7, p. 117.

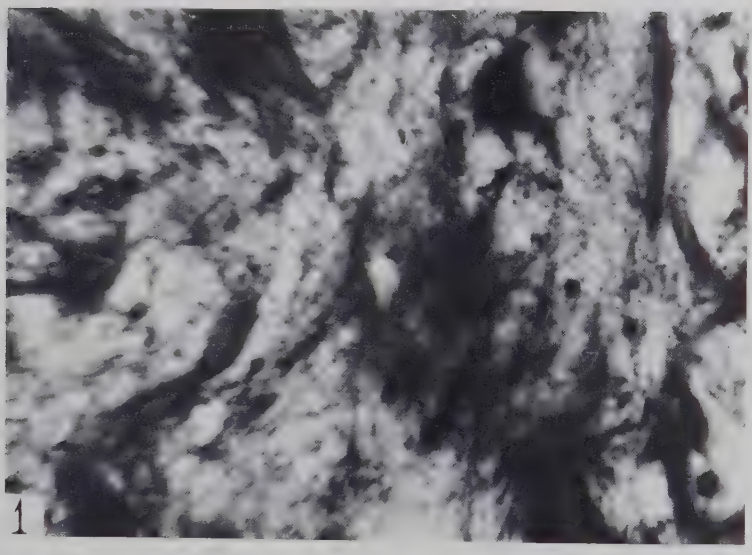
SCHMIEDEBERG, O. 1891 Ueber die chemishe Zusammensetzung des Knorpels. Arch. f. exp. Path. u. Pharm., Bd. 28, S. 355. Leipzig.

PLATE 1

EXPLANATION OF FIGURES

1 Photomicrograph of a section of fibrosis of a guinea pig's pancreas 14 days after duct ligation, impregnated with silver by the Foote method, showing the diffuse ground substance, stippled with silver, small reticular fibers, and the beginning formation of collagenic fibers and coincident 'spaces.'

2 Photomicrograph of a section of fibrosis of a guinea pig's pancreas 20 days after duct ligation, impregnated with silver by the Foote method, showing reticular fibers grouping into collagenic fibers, relative reduction in ground substance which appears only around these grouped fibers, and an increase in the 'spaces.' × 1122.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LITTER SIZE, BIRTH WEIGHT AND GROWTH TO WEANING IN THE CAT

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TWO FIGURES

In view of the wide use of the cat as an experimental animal it is surprising that its growth and related subjects of litter size and birth weight have been the subject of very few studies. Incidental data are reported by Abderhalden (1899), Thomas ('11), MacKay ('21) and v. Hoesslin ('26). Latimer and Ibsen ('32) have followed the growth of six male and six female kittens for 13 weeks. They noted that both sexes grow at about the same rate until the eighth week, after which the rate of growth of the males is much greater. Feeling the need for more accurate information concerning the growth of this animal, especially for comparison with that resulting from various experimental procedures, we have analyzed the data collected over several years from normal cats in our cat colony.

MATERIAL AND METHODS

Our material consists of thirty-three litters, in which there were 128 kittens. All of the kittens were born in the laboratory, some of the mothers having been bred there, others acquired while pregnant. All were of the usual mixed breeds obtained by random purchase. It is recognized that the variability found in such material is greater than would be expected had the animals been of a single stock. However, we

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feel that the data represent what is likely to be found in common laboratory practice and therefore of more value for use in control observations than would have been the case had they been of a single stock.

The colony is located in a well-lighted inside room. The pregnant mothers, and the mothers and kittens were kept in individual cages containing cheesecloth bedding, food pans and a sawdust pan. All were carefully cleaned daily. The food consisted of beef heart and milk, with occasional substitution of liver, in quantities yielding approximately 150 Calories per kilogram of combined weight of mother and kittens. In addition, each of the mothers was given 5 cc. of cod liver oil about once a week.

The kittens were weighed to the nearest gram once a day for the first 10 days and twice a week thereafter. When the kittens had attained an age of 50 days the mothers were removed from the cage. It was noticed that frequently the mothers had ceased to nurse the young from 7 to 10 days before this time and that manual expression of milk from the mammary glands had become impossible.

RESULTS

Litter size

In table 1 the distribution of the frequency of occurrence of litters of various sizes is set forth. The mean number of kittens was 3.88 per litter. Among the factors determining the size of the litter is the weight of the mother. Ibsen ('28) found in guinea-pigs a distinct positive correlation between the mother's weight with the number in the litter. Table 3 contains our data on this matter. The mean litter size for the thirteen smaller cats is 3.08 ± 0.19; for the ten larger, 5.00 ± 0.37. The difference is 4.64 times its probable error; accordingly, we are justified in concluding that the larger cats tend to have more kittens in the litter than do smaller cats. Concerning other factors which are important here, such as the age and hereditary characteristics of the mother and the ordinal rank of the litter, we, unfortunately, have no information.

Birth weight

The 'birth weight' here reported is taken on the morning following delivery. Since the kittens may have been as much as 20 hours old when first weighed and since kittens ordinarily grow about 10 gm. per 24 hours, an error of 10 gm. is possible in any one figure. However, the duration of gestation being variable by more than 1 day, no serious invalidation of our computation will arise from this source.

TABLE 1

Litter size and its influence on birth weight

NUMBER OF KITTENS	NUMBER OF LITTERS	MEAN BIRTH WEIGHT	P.E. OF MEAN	RATIO
		Gm.	*Gm.*	
1	1	102.	...	...
2	4	96.0	2.14	6.05
3	9	108.0	1.20	2.34
4	9	105.4	1.12	3.58
5	6	113.2	1.87	...
6	3	102.7	1.87	5.27
8	1	98.8	3.28	3.82

The ratio is: $\frac{\text{Birth weight in litter of five} - \text{birth weight of given litter size}}{\text{Probable error of above difference}}$

TABLE 2

Birth weight frequency

Interval, gm.	70–74	75–79	80–84	85–89	90–94	95–99	100–104	
Number of kittens	2	1	2	4	11	14	26	
Interval, gm.	105–109	110–114	115–119	120–124	125–129	130–134	135–139	140–144
Number of kittens	24	15	9	8	3	3	3	3

The birth weight of our kittens varied from 70 to 144 gm., the mean being 106.4 gm.; the standard deviation (σ), 13.5 gm.; the probable error of the mean, 0.81 gm.; and the coefficient of variability (Pearson's V), 0.127. The distribution of birth weights, grouped in 5-gm. intervals, is given in table 2.

Factors determining birth weight. Of the factors determining birth weight, our data enable us to analyze only two, the inflence of litter size and of the weight of the mother.

1. Influence of litter size. Among those studying the rodents, there is general agreement that the birth weight becomes progressively smaller as the litter size increases. The forms studied have been the guinea-pig (Minot, '91; Wright, '22; and Ibsen, '28); the rabbit (Hammond, '21; and Kopeć, '24); the albino rat (King, '15; and Angulo y Gonzalez, '32); and the mouse (Gates, '25). In the dog, at least to the extent that we can trust the scanty data of Simpson ('13), it appears that the middle-sized litters show smaller birth weights than do either smaller or larger litters. Our data on the cat agree with neither of the foregoing relations, but show that there is a distinct birth weight optimum when the litter contains five kittens, thus giving exactly the opposite picture to that in the dog. In view of this variation from the well-established and easily credible rule in the rodents, we have analyzed our data on this point statistically. F. W. Weymouth of this department has kindly calculated by the method of variance the chances that a difference between birth weights in relation to litter size as great as we have obtained could occur by random sampling. This proved to be less than 1 in 100. In order to test the validity of the existence of an optimum birth weight in litters of five, we have calculated the difference between mean birth weights of litters of various size and that of litters of five, and then determined the ratio of these differences to their probable errors. As may be seen from the data in table 1, the values for these ratios indicate that there is no reasonable doubt that the mean birth weights in litters of two, four, six and eight are smaller than that in litters of five, or, in other words, that a birth weight optimum has been satisfactorily demonstrated.

In attempting an explanation of the relations involved, it becomes necessary to consider separately the rise to, and the decline from, the optimimum birth weight with increasing

litter size. The latter phase is essentially the same problem as has been confronting the students of rodents: Minot (1891) considered that larger litters induced earlier parturition, so cutting short the period of prenatal growth; however, Angulo y Gonzalez ('32) and Ibsen ('28) have shown that even at similar conception ages the fetuses of larger litters are smaller. Ibsen recognized two factors: crowding of the uterus in larger litters, and a limitation on the capacity of the maternal organism to supply nutrition adequate for optimal growth of a larger number of fetuses. Hammond ('21) denies for the pig any deleterious effect of uterine crowding. However, other factors must be invoked to explain the rise to the optimum.

If the small size of litters produced by certain mothers be the result of a general debility (immaturity, chronic malnutrition, etc.), which might decrease the number of ovulations, and increase the embryonic and fetal mortality, such debility might also express itself in poor nutrition and consequent smaller birth weight of the kittens. In this event, litter size and birth weight would increase together in mothers of progressively better physical condition. However, the fact that the birth weight tends to be greater when the mothers are smaller than when they are larger speaks against the explanation just given. Further, if true, it must be a peculiarity of the cat among the species so far studied, for no such relations have been reported for the rodents.

Another explanation of these relations might be in a lack of genetic homogeneity in our material, such as the existence of two strains, one tending to have smaller litters of kittens of lower birth weight, and the other to have larger litters of kittens of higher birth weights, in both strains the birth weights decreasing as the litter size increased. Averaging the litters of the same number of kittens in a mixture of such strains would reproduce our data. However, frequency curves of birth weights at the various litter sizes are never clearly bimodal. Further, if such strains exist, they are not related to the weight of the mothers, for the relations re-

ported hold equally well (as far as the data permit judgment) for the kittens of the lighter half of the mothers and for the kittens of the heavier half of the mothers as for the group as a whole. See figure 1.

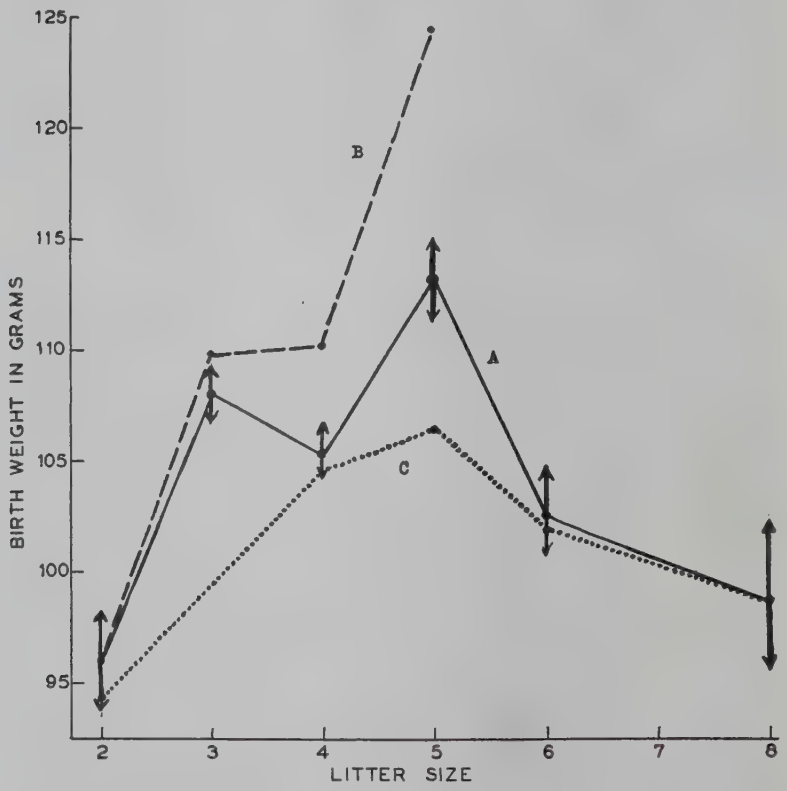

Fig. 1 Relation of mean birth weight to litter size in the cat. A is based on all 128 kittens, the arrows representing the probable error of the mean, added and subtracted from the mean. B is based on thirty-nine kittens, the mothers of which weighed from 2.38 to 3.45 kg. C is based on forty-eight kittens, the mothers of which weighed from 3.54 to 6.00 kg.

2. Influence of the weight of the mother. In the albino rat, King ('15) observed that the birth weight of the young was proportional to the body weight of the mother, although she regarded the relation as not due to the weight of the female

itself, but to the factors on which the weight largely depend, i.e., age and physical condition. Ibsen ('28) found the same empirical relation in guinea-pigs, as did Hammond ('21) in rabbits. In table 3 is presented our data relative to this problem. Again, the cat differs from the rodents, for there appears to be a strong tendency for small mothers to have large kittens. The mean birth weight of the kittens of the thirteen smallest mothers is 110.0 $\pm$ 1.59 gm.; while that of the kittens of the ten largest mothers is 102.5 $\pm$ 0.36 gm. The ratio of the difference in birth weights of the two groups

TABLE 3

Influence of weight of mother on litter size and birth weight

WEIGHT INTERVAL (AND MEAN)	NUMBER OF LITTERS	NUMBER OF KITTENS	KITTENS PER LITTER	MEAN BIRTH WEIGHT	MEAN TOTAL LITTER WEIGHT PER KILOGRAM MOTHER
Kg.				*Gm.*	*Gm.*
2.381–2.977 (2.747)	6	20	3.3	108.3	131.4
3.119–3.420[1] (3.333)	6	19	3.5	111.8	105.6
3.544–3.856 (3.634)	5	22	4.4	101.3	122.8
4.018–6.000 (4.901)	5	28	5.6	103.4	119.0

[1] The single case in which a litter of one occurred has been eliminated from this group. The weights given are those after delivery.

to its probable error is 4.66, which shows that the difference is significant.

The tendency of small mothers to have larger kittens stands opposed to their tendency to have fewer kittens; however, the latter tendency is more marked, since the mean litter weight per kilogram of mother's weight is more nearly constant than the mean litter weight itself.

Growth to weaning

The data on the growth of kittens up to the fiftieth day has been obtained on a group of seventy kittens. In order to

eliminate kittens which were not in sound health, all those individuals whose growth curves showed for any day a value for body weight lower than that 10 days previously were dropped. This criterion checked well with clinical manifestations: appetite, activity, condition of the fur, etc.

Growth curve of the group. In figure 2 there is plotted the mean weights of the seventy kittens at 5-day intervals up to the age of 50 days. The growth curve approximates closely to a straight line over this period. In the period from birth to 15 days there is a gradual reduction in the slope, while from 15 to 35 days the curve is almost perfectly linear. There appears to be a significant acceleration during the last 15 days. That this increase in growth rate cannot be attributed to the beginning of ingestion of solid food by the kittens is shown by the fact that they were observed to do this almost invariably between the twenty-fifth and thirtieth days. Since the percentage rate of growth did not increase, this upturn may be attributed simply to the larger mass of growing tissue, rather than a specific acceleration in the growth processes. The course of growth agrees well with that reported by Latimer and Ibsen ('32) on their twelve kittens, except that their animals showed relatively slow growth in the first week —a time at which ours were gaining most rapidly.

In common with these authors, we have failed to find any evidence for a postnatal decrease in weight. If such occurs in the cat, it is more than made up within a few hours after birth.

Variability in growth. The standard deviations (σ) and the coefficient of variability ($V = \frac{\sigma}{m}$) have been computed by the conventional methods and are set forth in table 4. The variability is least at birth, increases gradually to a maximum at 40 days and then apparently decreases slightly. The values are of the same order of magnitude as those found for the rat by Jackson ('13) and by King ('23), and for human infants by Pearson (1899).

Growth of a group given particular care. After the termination of the observations on the greater part of the material,

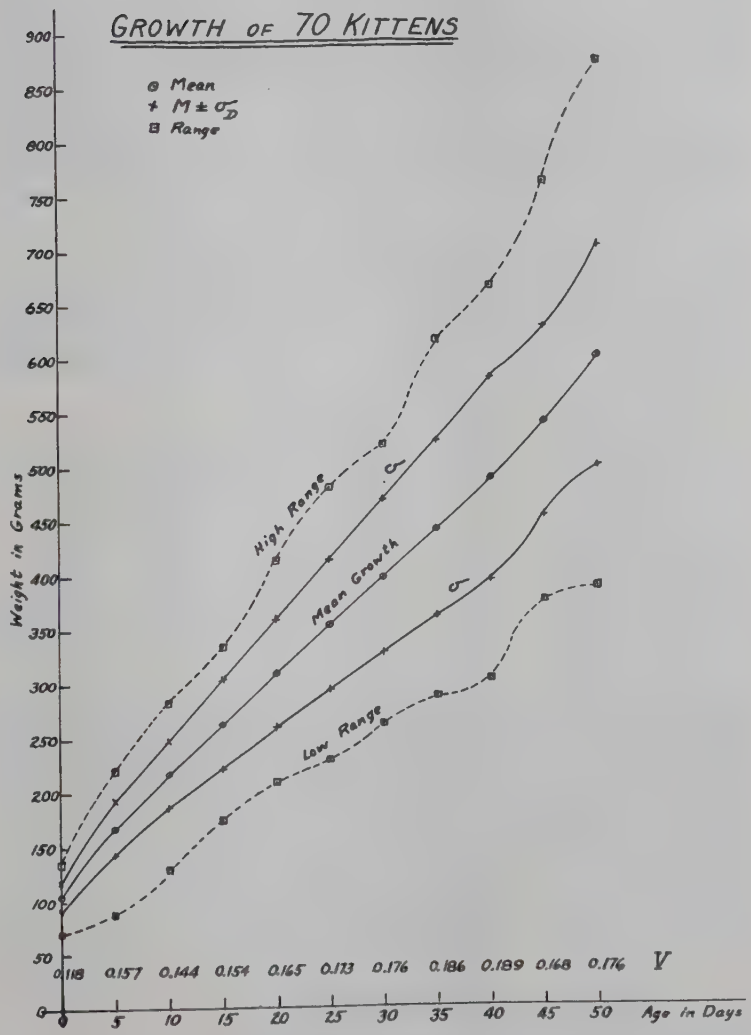

Fig. 2 Growth of kittens during the first 50 days of life.

a smaller group of animals, fourteen kittens in six litters, were studied with particular care; they were weighed daily, kept scrupulously clean, protected from temperature extremes and given a diet designed to meet all known dietary requirements. The diet consisted of 67 gm. of ground lean beef heart and 118 cc. pasteurized whole milk per kilogram of combined weight of mother and kittens. This diet yielded, according to the tables of Atwater and Benedict ('06), 250 calories per

TABLE 4

Body weight and variability up to weaning

AGE	MEAN WEIGHT	STANDARD DEVIATION	RANGE		PER CENT GROWTH PER DAY	COEFFICIENT OF VARIABILITY
			Min.	Max.		
Days	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent*	
0	105	14	70	135	...	0.118
5	167	25	89	221	7.4	0.157
10	217	31	130	284	4.6	0.144
15	264	42	175	335	3.5	0.154
20	310	50	209	415	2.9	0.165
25	355	60	230	482	2.5	0.173
30	398	70	264	521	2.1	0.176
35	442	82	289	619	1.9	0.186
40	490	94	305	668	2.1	0.189
45	543	88	378	765	2.0	0.168
50	604	102	390	876	2.0	0.176

kilogram of cat, 23 per cent as protein, 68 per cent as fat and 9 per cent as carbohydrate. To insure adequacy in mineral constituents, 20 mg. of the following salt mixture were added to each kilogram of meat:

	gm.
NaCl	38.0
Mg citrate	32.5
KH_2PO_4	12.2
$CaHPO_4.2H_2O$	7.8
KCl	7.0
Ferric citrate	1.8
KI	0.5
$CuCl_2$	0.005
$MnCl_2$	0.005
	99.81

The copper was added to avoid any nutritional anemia such as is reported by Hart, Steenbock, Waddell and Elvehjem ('28), and the manganese to prevent any loss of maternal care of the young such as that observed by Kemerer, Elvehjem and Hart ('31). To assure adequacy in respect to vitamines, approximately 130 gm. of lettuce were ground up with each kilogram of meat. On every other day each cat received 14 gm. of dried yeast mixed with a small portion of meat, while on alternate days she was given 1 cc. of a 2:1 mixture of cod liver oil and corn oil (Mazola) per kilogram of cat. The cats received meat once a day, milk twice. Food not eaten within 3 hours was removed. The animals ordinarily consumed the rations quickly and completely.

TABLE 5

Influence of birth weight on rate of growth

BIRTH WEIGHT RANGE	NUMBER OF KITTENS	MEAN BIRTH WEIGHT	MEAN WEIGHT AT 25 DAYS	RATIO: 25 DAYS / BIRTH	MEAN WEIGHT AT 50 DAYS	RATIO: 50 DAYS / BIRTH
Gm.		*Gm.*			*Gm.*	
70–98	18	91.2	318.0	3.48	543.3	5.96
100–104	17	101.9	350.5	3.44	609.5	5.98
105–112	17	108.0	359.9	3.34	634.1	5.87
113–136	18	120.3	398.7	3.31	641.6	5.33

In spite of the improved conditions, the growth rate of the kittens in this group did not differ significantly from that of the earlier group. The variability of this group is also closely similar to that of the larger group up to 30 days; thereafter, however, the variability of the earlier group is markedly greater. It appears that the care given the smaller group did not cause them to approach an optimal growth more closely, but did cause the individuals to grow in a more nearly uniform manner.

Factors influencing the rate of growth

1. Birth weight. In table 5 the seventy kittens used in the growth study are divided into four groups, approximately quartiles, on the basis of birth weight, and the relative growth

(ratio to birth weight) computed at 25 and 50 days of age. It appears that, while the smaller kittens grow somewhat more rapidly than the heavier ones up to 25 days of age, this is not sufficient to offset the initial advantage of the latter. The relations from 25 to 50 days are less regular, but still leave those heavier at birth ahead of the lighter. Kopeć ('32) found similar relations in the early growth of the mouse, as did King ('16) of the rat. Hammett ('18) calculated that the per cent increase in body weight of human infants during the first 13 days of life was inversely proportional to their birth weights.

2. Litter size, according to our data, appears to have little influence on the rate of growth of kittens. The literature on this matter is reviewed by Kopeć ('32).

3. The weight of the mother does not appear to be clearly related to the rate of growth of kittens, although there may be a tendency for the kittens of middle-sized mothers to have the advantage.

SUMMARY

The mean litter size in the domestic cat is 3.88 kittens, the larger cats tending to have more kittens in the litter. The mean birth weight of kittens born in litters of five is significantly greater than that of kittens born in smaller or larger litters. This relation appears to be peculiar to the cat, at least in the forms so far studied. The birth weight of kittens born of smaller mothers is significantly greater than that of kittens born of larger mothers. This is the converse of the relation reported for rodents.

The growth curve of kittens is practically linear up to the fiftieth day of life. Its variability is comparable to that observed for other forms. The rate of growth is somewhat faster in kittens below average in birth weight, but does not seem to be clearly related to either litter size or weight of the mother.

LITERATURE CITED

ABDERHALDEN, E. 1899 Die Beziehung der Wachstumsgeschwindigkeit des Säuglings zur Zusammensetzung der Milch beim Kaninchen, bei der Katze und beim Hunde. Zeitschr. physiol. Chem., Bd. 26, S. 487.

ANGULO Y GONZALEZ, A. W. 1932 The prenatal growth of the guinea-pig. Anat. Rec., vol. 52, p. 117.

ATWATER, W. O., AND A. P. BRYANT 1906 The chemical composition of American food materials. U. S. Dept. of Agric. Bull. no. 28.

GATES, W. H. 1925 Litter size, birth weight and early growth rate of mice. Anat. Rec., vol. 29, p. 183.

HAMMETT, F. S. 1918 The relation between growth capacity and weight at birth. Am. J. Physiol., vol. 45, p. 396.

HAMMOND, J. 1921 Further observations on the factors controlling fertility and foetal atrophy. J. Agric. Sci., vol. 11, p. 337.

HART, E. B., H. STEENBOCK, J. WADDELL AND C. A. ELVEHJEM 1928 Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. J. Biol. Chem., vol. 77, p. 797.

v. HOESSLIN, H. 1926 Wachstumversuche an Katzen. Zeitschr. Biol., Bd. 85, S. 248.

IBSEN, H. L. 1928 Prenatal growth in guinea-pigs with special reference to environmental factors affecting weight at birth. J. Exp. Zoöl., vol. 51, p. 51.

JACKSON, C. M. 1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. J. Anat., vol. 15, p. 1.

KEMERER, A. R., C. A. ELVEHJEM, AND E. B. HART 1931 Studies on the relation of manganese to the nutrition of the mouse. J. Biol. Chem., vol. 92, p. 623.

KING, H. D. 1915 On the weight of the albino rat at birth and the factors that influence it. Anat. Rec., vol. 9, p. 213.

———— 1916 On the postnatal growth of the body and of the central nervous system in albino rats that are undersized at birth. Anat. Rec., vol. 11, p. 41.

———— 1923 The growth and variability in the body weight of the Norway rat. Anat. Rec., vol. 25, p. 79.

KOPEĆ, S. 1924 On the influence exerted by certain factors on the birth weight of rabbits. Anat. Rec., vol. 27, p. 95.

———— 1932 Die prozentuelle Wachstumsgeschwindigkeit der Mäuse in Bezug auf das Gewicht der Neugeborenen. Arch. f. Entwicklungsmek., Bd. 126, S. 575.

LATIMER, H. B., AND H. L. IBSEN 1932 The postnatal growth in the body weight of the cat. Anat. Rec., vol. 52, p. 1.

MACKAY, H. M. M. 1921 Effect on kittens of a diet deficient in animal fat. Biochem. J., vol. 15, p. 19.

MINOT, C. S. 1891 Senescence and rejuvenation. I. On the weight of guinea-pigs. J. Physiol., vol. 12, p. 97.

PEARSON, K. 1900 Data for the problem of evolution in man. III. On the magnitude of certain coefficients of correlation in man, etc. Proc. Roy. Soc., vol. 66, p. 23.

SIMPSON, S. 1913 The rate of growth in the dog. Proc. Soc. Exper. Biol. Med., vol. 10, p. 97.

THOMAS, K. 1911 Über die Zusammensetzung von Hund und Katze während der ersten Verdoppelungsperioden des Geburtsgewichtes. Arch. Anat. Physiol. (Phy. Abt.), S. 9.

WRIGHT, S. 1922 The effects of inbreeding and cross breeding on guinea-pigs. U. S. Dept. of Agric. Bull. no. 1090.